Are circulating hematopoietic stem and progenitor cells a potential biomarker for therapy response and disease progression in patients with squamous cell carcinoma of the head and neck?

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We hypothesize that the cytokines secreted by HNSCC may perturb the hematopoiesis in patients. We wonder whether this perturbation can be restored after local treatment of the tumor.Primary objective: In this pilot study, we aim to prospectively…

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Respiratory and mediastinal neoplasms malignant and unspecified
Study type	Observational invasive

Summary

ID

NL-OMON46829

Source
ToetsingOnline

Brief title
CHASE-study

Condition

- Respiratory and mediastinal neoplasms malignant and unspecified

Synonym
Squamous cell carcinoma of the head and neck; head and neck cancer

Research involving
Human

Sponsors and support

Primary sponsor: Antoni van Leeuwenhoek Ziekenhuis
Source(s) of monetary or material Support: Stichting Virtutis Opus en de W.M. De Hoop Stichting.

Intervention

Keyword: hematopoietic stem and progenitor cells squamous cell carcinoma of the head and neck, HSPC, SCCHN

Outcome measures

Primary outcome

Main study parameter/endpoint

* Cytokine detection in pre- and posttreatment peripheral blood samples (26ml) from HNSCC patients will be performed and compared with results obtained in blood samples from healthy volunteers. In case of HNSCC recurrence, we will repeat this analysis on a newly obtained blood sample. Depending on the number of cytokines, optimal detection limits and operability, we will choose between BD* Cytometric Bead Array (at NKI-AVL) and Luminex® Multiplex Assay. The Luminex® analysis will be performed at UMCU.
* Pre- and posttreatment blood from HNSCC patients, as well as healthy control blood samples, will be analyzed with flow cytometry in order to characterize the composition and frequency of HSPCs as well as their downstream progenitor and differentiated cells. In case of HNSCC recurrence, we will repeat this analysis on a newly obtained blood sample.
* Fresh tumor will be obtained from the surgical specimen in patients who will undergo an operation as standard of care. These will be used for the production

of tumor supernatant and tumor pieces, cytokine detection, flow cytometry and cryopreservation.

* One or two sections of FFPE pretreatment tumor biopsies, obtained for routine diagnostic purposes, will be stained for the identification of HSPCs, downstream progenitor and differentiated cells using immunohistochemistry and immunofluorescence. In case of HNSCC recurrence, we will repeat these analyses on a newly obtained tumor biopsy (taken for diagnostic purposes).

* During the routine biopsy procedure (in case of primary HNSCC or recurrence), patients will be requested (non-mandatory) to donate an additional, non-mandatory tumor 5mm biopsy for cytokine detection, flow cytometry and cryopreservation in order to run future analyses.

* Clinical parameters such as disease status and survival data (disease-specific and overall) will be collected for up to two years after treatment.

Secondary outcome

Once the correlation between the responding progenitor populations and their corresponding lineage cells is made based on previous results.

1. Isolate all corresponding progenitor populations from healthy donor (obtained from Sanquin) and set up an in vitro culture system. By manipulating the cytokine combination (hints got from part I and II) in the medium, study whether the changes of composition and differentiation of certain progenitor populations recapitulate what happens in patients with HNSCC.

2. Culture corresponding progenitor populations from healthy donor, with or

without the medium supernatant of HNSCC tumor, then compare the composition and differentiation of the progenitor populations cultured with different conditions. With this approach, the responding progenitor population(s) will be detected.

3. Isolate the responding progenitor populations from the peripheral blood of HNSCC patients and set up an in vitro culture system. By manipulating the cytokine combination in the medium, study whether the changes of composition and differentiation of the responding progenitor populations recapitulate what happens in patients after therapy.

4. Culture the responding progenitor populations from the peripheral blood of HNSCC patient alone, or with the autologous tumor pieces/cells, or with the medium supernatant of the autologous tumor, then compare the composition and differentiation of the responding progenitor populations cultured with different conditions.

Study description

Background summary

Hematopoietic stem cells and their downstream specialized progenitor cells (HSPCs) are responsible for the renewal of blood cells. Although bone marrow is the principle resident site of HSPCs, they are also present in blood and other tissues. Small numbers of HSPCs continually egress from bone marrow into blood, circulate throughout the body and back to the blood via lymph1. HSPCs cannot only replace progeny pools, but also act as pivotal primary immune responders to various pathological conditions. For example, HSPCs can remain in infected tissue and differentiate to replenish local supplies of immune cells1.

Cancer is associated with profound perturbations in hematopoiesis as well. Wu et al. reported that the composition of circulating HSPCs was significantly altered in patients with breast cancer, lung cancer, esophageal cancer,

gastrointestinal cancer, hepatocellular carcinoma, ovarian cancer, and cervical cancer2. The frequencies of circulating granulocyte-monocyte progenitors (GMPs), a highly proliferative subset of HSPCs with committed lineage potential, were increased four- to sevenfold in all types of tumors examined. Furthermore, the circulating hematopoietic precursors exhibited myeloid bias with a skew toward granulocytic differentiation in patients with solid tumors. More importantly, these myeloid precursors were found to be selectively enriched in tumor tissues and positively correlated with disease progression2. Adding granulocyte colony-stimulating factor (GM-CSF) and IL-6, substances produced by many solid tumors, to an in vitro culture of umbilical cord blood-derived HPSCs promoted GMP expansion and myeloid-derived suppressor cell (MDSC) differentiation, which recapitulated the in vivo observation in patients with solid tumors2. MDSCs are key regulators of immune dysfunctions. They can enhance the *stemness* of cancer cells, facilitate tumor progression, metastasis and angiogenesis3-6.

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world. Despite significant advances in the treatment modalities involving surgery, radiotherapy, and concomitant chemoradiotherapy, the 5-year survival rate remained below 50% for the past 30 years. This poor prognosis is likely associated with the fact that HNSCC suppresses the host immune system in several ways7. It has also been reported that the percentage of both monocytic-MDSCs (M-MDSCs) and granulocytic-MDSCs (G-MDSCs) are increased in the peripheral blood of patients HNSCC8. Furthermore, the level of circulating G-MDSCs significantly correlated with the tumor stage and the overall survival rate of HNSCC patients8. However, the connection between MDSCs and circulating HSPCs has not been investigated yet for HNSCC.

Cancer and the immune system interact with each other throughout the process of tumorigenesis, progression and metastasis, which influences the therapy response and clinical outcome. Since HSPCs possess the most plastic and proliferative potential, patrol constantly via peripheral blood and act as primary responders to cancer, investigating the frequency and composition of circulating HSPCs and their commitments for differentiation will help us understand their role in immunopathogenesis of HNSCC.

Several studies have suggested that the level of immune inhibitory CD34+ progenitor cells was associated with GM-CSF secretion by the tumor and was elevated in both the peripheral blood9 and tumors10 of HNSCC patients when compared to healthy controls. Furthermore, higher levels of GM-CSF and CD34+ cells correlated with more advanced disease and nodal involvement, and depletion of CD34+ cells in disassociated cancer cell suspension lead to increased IL-2 production by intratumoral T cells9,10. However, since these studies were performed before advanced multicolor flow cytometry was developed, the so-called CD34+ progenitor cells were not phenotypically described in detail. Actually, researchers only used Lin*CD34+ as definition markers, which are far from adequate to tell the identity of these cells. Although some

studies showed that under certain in vitro cytokine treatments, these *CD34+ progenitor cells* can differentiate into myeloid cells and even into dendritic cells (DCs) with antigen-presenting capability9,11, poor population purity has dampened the credibility of these findings. Hence, the frequency, composition and clinical value of circulating HSPCs in HNSCC patients need to be reassessed in more detail.

Hematopoiesis is tightly regulated by a number of circulating hematopoietic growth factors, which can also be secreted by solid tumors. In a previous study involving solid tumors other than HNSCC, tumor-secreted GM-CSF and IL-6 could induce the differentiation of HSPCs into MDSCs2. We therefore hypothesize that some cytokines produced by HNSCC may influence the hematopoiesis in patients. Considering the increasing MDSCs in patients with HNSCC, we speculate that the hematopoiesis might be altered with a myeloid bias and a skew towards granulocytic differentiation. Thus, we hypothesize that the perturbation of hematopoiesis by HNSCC can be restored by removing the tumor. In order to answer this question, we will investigate the hematopoiesis of HNSCC patients before and after local treatments, which are surgery and/or radiotherapy. Since systemic chemotherapy may cause myelosuppression or egress of the HSPCs from bone marrow, the present study excludes the patients receiving concomitant chemotherapy or other medication known to affect immune system, except for patients who undergo immunotherapy with immune checkpoint inhibitors prior to surgery or radiotherapy.

Study objective

We hypothesize that the cytokines secreted by HNSCC may perturb the hematopoiesis in patients. We wonder whether this perturbation can be restored after local treatment of the tumor.

Primary objective: In this pilot study, we aim to prospectively investigate the frequency, composition and differentiation commitments of circulating and tumor-resident HSPCs in treatment-naïve HNSCC patients, and compare them with the results from healthy controls. Next, we aim to assess whether treatment consisting of surgery, immunotherapy, radiotherapy or a combination of these modalities could restore the perturbed hematopoiesis by comparing the sample profile before and after treatment. We will correlate our findings to clinical parameters such as disease progression and 2-year disease specific and overall survival data.

Secondary objective: We will set up an in vitro culture study aiming to investigate the mechanism of altered HSPC composition, frequency and differentiation in patients with HNSCC when compared to healthy, age-matched controls. Additionally, we aim to check whether we can experimentally recapitulate the findings we observed in patients with HNSCC.

Study design

This is a single-center, prospective study. 30 patients who meet all relevant criteria will be included. Patients will be asked to donate 26ml blood for study purposes at baseline, if possible during a routine blood draw. During a routine biopsy procedure (in case of primary HNSCC or recurrence during 2 years follow up), patients will be asked to donate an additional, non-mandatory 5mm tumor punch biopsy for cytokine detection, flow cytometry and cryopreservation in order to run future analyses. Hereafter, patients will undergo standard-of-care treatment consisting of surgery and/or radiotherapy. Ten patients who receive neoadjuvant immunotherapy as a consequence of participation in the N16IMC trial will be included. All patients will enter regular post-treatment follow-up, as deemed appropriate by the Head and Neck Oncology board or, for immunotherapy patients, as described in the N16IMC protocol. For the purpose of this study, survival and disease progression data will be collected for up to two years after treatment.

Five weeks after neoadjuvant immunotherapy and ten to twelve weeks after surgery or (postoperative) radiotherapy, another 26ml of blood will be drawn. In the case of recurrent disease after treatment, another venous puncture will be performed during which additional 26ml will be requested for study purposes.

As a control group, we aim to analyze blood samples from 20 healthy, age-matched controls. To obtain these, the researchers will approach potential volunteers from within their patients will be kindly requested to bring a healthy friend or relative of matched age who is willing to offer a control blood sample. If this proves unfeasible, the investigators will complement the control sample pool by asking blood from their own age-matched colleagues, friends and relatives.

Study burden and risks

Risk
Participating patients will be asked to donate an additional 26ml of blood during routine venous puncture performed for diagnostic purposes. No morbidity is expected from this procedure Five weeks after neoadjuvant immunotherapy, ten to twelve weeks after surgery and/or radiotherapy and in the case of HNSCC recurrence, patients will be asked to donate 26ml once again. Wherever possible, this will be planned to coincide with a venous puncture performed for diagnostic/follow-up purposes, as to minimize patient discomfort. Since all necessary patient tumor tissues will be obtained from the surgical specimen in collaboration with the pathologist, there will be no added burden for the patient. A non-mandatory, additional biopsy will be requested from patients, preferably taken during routine biopsy for diagnostic purposes. Apart from slight pain and discomfort, no additional morbidity is expected from this biopsy.

Benefit
There are no direct benefits for our participants.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:;* Have a primary mucosal HNSCC (T1-4, N0-3, M0) of any head and neck anatomic subsite. Patients with recurrent HNSCC are eligible only if they have been disease-free *5 years before enrolment.
* Be eligible for curative treatment with surgery, radiotherapy, immunotherapy or a combination of these modalities.
* Screening laboratory values must meet the following criteria: WBC * 2.0x109/L, Neutrophils * 1.5x109/L, Platelets * 100 x109/L, Hemoglobin * 5.5mmol/L, Creatinine * 1.5x ULN, AST * 3

x ULN, ALT * 3 x ULN, Total Bilirubin *1.5 X ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL).

Exclusion criteria

* HNSCC patients treated with concomitant chemoradiation therapy.
* The presence of any malignancy other than the present HNSCC, unless:
o It has been curatively treated *5 years prior to first study-related blood collection;
o It is a curatively treated cutaneous basal cell carcinoma, cutaneous squamous cell carcinoma, carcinoma in situ of the breast or carcinoma in situ of the bladder.
* The presence of active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have:
o Vitiligo;
o Type I diabetes mellitus;
o Residual hypothyroidism due to an autoimmune condition, only requiring hormone replacement;
o Psoriasis not requiring systemic treatment;
o Conditions not expected to recur in the absence of an external trigger.
* The presence of a condition requiring systemic treatment with either corticosteroids (>10mg daily prednisone equivalents) or other immunosuppressive medications.
* The presence of active hepatitis B, hepatitis C, active tuberculosis, syphilis, HIV or AIDS.
* The presence of any disease or use of medication which, in the investigators* opinion, might significantly influence hematopoiesis.

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)

Primary purpose: Diagnostic

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	13-08-2018
Enrollment:	30

Type:	Actual

Ethics review

Approved WMO Date:	19-06-2018
Application type:	First submission
Review commission:	METC NedMec
Approved WMO Date:	26-07-2018
Application type:	Amendment
Review commission:	METC NedMec

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL64935.031.18