Final Study Report

Study Title: Combined hypofractionated stereotactic body radiotherapy with immunomodulating systemic therapy for inoperable recurrent head and neck cancer: detection of the maximum tolerated dose.

EudraCT number: 2017-000133-31

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Study protocol code: AGO/2017/001

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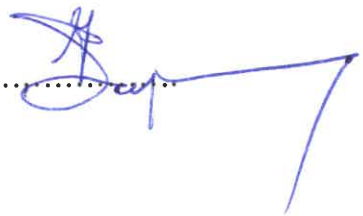
Date of report: 22/01/2021

Name and signature Sponsor:

Date signature Sponsor: 22-JAN-2021 

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1. Introduction

The standard treatment in inoperable locally or regionally recurrent head and neck cancer has long been palliative systemic therapy using the so-called EXTREME-scheme: a combination of cisplatin, 5-fluorouracil and cetuximab. This therapy remains without realistic chances of cure [Vermorken et al., 2008]. More recently, immunotherapy using nivolumab has demonstrated to result in long-term disease control of 1-2 year in cisplatin-refractory recurrent or metastatic head and neck cancer, however only in a small portion of patients (13%) [Ferris et al., 2016].

High-dose fractionated re-irradiation using IMRT or a comparable conformal photon technique can be offered as an alternative for patients with inoperable locoregional recurrent disease in absence of distant metastases, with relatively low but existing chances for disease control at the cost of severe late toxicity [Duprez, 2014; Haraf, 1996; Hoebers, 2011; Spencer, 2008; Platteaux, 2011]. The longer the time interval between first therapy and recurrence, e.g. > 2 years, the better the chances for cure will be. Patients with a shorter time period, i.e. < 1-2 year between a first and second treatment with radiotherapy have the worst prognosis and will probably only have a short period of disease control [Duprez, 2009: Ward, 2018].

Fractionated high-dose local or regional re-irradiation is mostly given in a 6-7 weeks scheme. A considerable part of these patients will succumb early due to disease progression. This 6-7 weeks scheme costs a valuable part of the patient's remaining lifetime and an even longer period of acute radiation-induced toxicity is often observed. Moreover, unacceptable late normal tissue toxicity can be a matter of concern in re-irradiated patients due to high cumulative radiation doses [Kao, 2003; Duprez 2014]. One of the most devastating complications is carotid artery blow-out, mostly resulting in dead. This fatal side-effect is mostly associated with skin invasion by tumor, soft tissue necrosis in loco of involvement of a carotid artery by > 180° [Lartigau 2013, Yamazaki 2015].

Using stereotactic body radiotherapy (SBRT), high radiotherapy doses can be given in a short time span. Severe late adverse events have been reported using SBRT but seem less frequent than in patients re-treated with conventional schedules. However, the dose that we can give using SBRT for reirradiation is limited; single fraction doses of 10–13 Gy or higher should be avoided [National Cancer Action Team 2011]. Comparative analysis of SBRT vs. fractionated high-dose reirradiation for recurrent head and neck cancer also demonstrated to result in comparable outcome and toxicity for patients with worse prognosis, excluding the indication of SBRT for patients with the best prognosis (> 2 years interval or absence of organ dysfunction) [Vargo 2018].

A possible solution to be able to administer higher doses is combining SBRT with dose painting, thus giving these high doses on small subvolumes only. Targeting the tumor using dose painting by numbers allows to deliver very high doses in subvolumes of the tumor that are hypothesized to be the most radioresistant, while limiting the dose in surrounding tissues [Duprez, 2011; Vanderstraeten, 2006; Vanderstraeten, 2006']. Especially in recurrent head-and-neck cancer it makes sense to combine high fraction doses to parts of the tumor that exhibit high metabolism with lowered doses in the surrounding normal tissues. FDG-signal intensity correlated with a variety of factors including inflammation of the tumor micro-environment and cancer cell density. Inflammation acts pro-proliferative, pro-invasive, pro-metastatic, pro-angiogenic and anti-apoptotic and is most efficiently targeted by high doses per fraction. The rationale to target regions of high cancer cell densities lays in the release of cancer-specific antigens that are at the origin of selective anti-tumor immune response.

Addition of concomitant therapy to reirradiation may further improve outcomes due to radiosensitization and direct cytotoxicity [Kao, 2003]. Therefore we aim to combine high doses with concomitant therapy in the proposed study.

The immunomodulatory effect caused by radiation has been demonstrated both in animal models and clinical trials and leads to an enhanced local control as well as to eradication of distant metastasis [De Meerleer, 2014; Formenti, 2012; Popp, 2016]. This so-called abscopal effect is reached through a systemic immune response evoked by the release of damage-associated molecular patterns (DAMPs) by the dying tumor-cells, also called immunogenic cell death (ICD) [Lauber, 2012].

Since some time, it has become clear that the therapeutic potential of radiation does not only exist of local and direct cytotoxic but also indirect local cytotoxic and long-range abscopal (systemic) effects

[Lauber, 2012; Formenti 2009; Formenti 2012]. During their cell-death process malignant cells exposed to radiation can release DAMPs, which induce recruitment of antigen-presenting cells (APCs) and eventually lead to ICD. This process is most probably not apoptotic (until recently assumed to be the most prominent cell death caused by radiation) but rather a regulated necrotic (RN) cell death such as necroptosis or ferroptosis [Vanden Berghe, 2014]. In this way irradiated cells can, at least in some circumstances, prime a tumor-specific immune response which targets local malignant cells that survived radiation as well as lead to a systemic immune response [Lauber, 2011]. Also chemotherapeutic agents such as doxorubicin, oxaliplatin, mitoxantrone and cyclophosphamide are known immunomodulators who can induce ICD [Galluzzi, 2012]. The combination of both immunomodulatory drugs and radiation, can lead to an enhanced ICD in-vitro when used concurrently [Golden, 2014]. Although the evidence to support this finding in clinical setting is scarce, ICD can at least partly explain the success of concurrent chemoradiation treatment regimens, for example in head-and-neck cancer [Suzuki, 2012].
The (chemo)radiotherapy-produced proimmunogenic effects, which are often masked by the overwhelming immune-suppressive microenvironment that characterizes cancers, can be brought to the front by combining immune check-point inhibitors (anti-CTLA4 and/or anti-PD(L)-1), e.g. nivolumab, pembrolizumab and durvalumab [Dewan, 2012; Golden, 2013; Postow, 2012; Popp 2016].
We hypothesize that an abscopal effect could be present for patients presenting locoregional recurrent disease with asymptomatic distant metastases, thereby offering at least symptom control at the primary site while palliative systemic treatment could be postponed [Formenti, 2009;].

References: see under 4.5.

1. Objectives of the study

1.1 Primary objectives

To derive the maximum tolerated dose of hypofractionated stereotactic body radiotherapy (SBRT) using dose painting by numbers with immunomodulating systemic therapy in patients that are reirradiated for recurrent squamous cell carcinoma of the head and neck.

1.2 Secondary objectives

To assess symptom palliation
☐ To assess local control.
☐ To estimate overall and progression-free survival.
☐ To estimate grade ≥ 3 toxicity-free survival
☐ To assess quality-of-life (QOL)
☐ To assess the topographic distribution of recurrence (inside/outside FDG-avid GTV)
☐ To assess time to further treatment
☐ To assess the immune response

2. Investigational Medicinal Product

1 Nivolumab
Considered as standard therapy in patients with cisplatin refractory locoregional disease recurrence. Nivolumab will be administered as per current standard of care. In case patients that are treated with nivolumab will be included in the trial, they will not be treated with cyclophosphamide.

2 Cyclophosphamide
2.1 Composition and dosing

Endoxan 50 mg tablets.

2.2 Producer

Baxter.

2.3 Distributor

- Hospital pharmacy, UZ Gent, C. Heymanslaan 10, 9000 Gent
- Hospital pharmacy, UZ Leuven
- Hospital pharmacy, CHU-UCL Namur, site Sainte-Elisabeth.

2.3 Packaging

Commercial package, labeled according to ICH-GCP-labeling criteria.

2.4 Administration way

Patients will take cyclophosphamide orally 50 mg tablets, 1 tablet a day from the first day of irradiation for 8 consecutive weeks. Patients must be instructed to obey to following guidelines:

- Intake of grapefruit and grapefruit juice is forbidden during the trial
- avoid intake of alcohol
- the tablets must be swallowed as a whole; they may not be smashed.
- to drink at least 2 liters of water per day
- the tablets would best be taken during breakfast
- patients must try to take it at the same time every day.
- Take contact whenever they have signs of infection

Following blood values needed to be checked every week while under treatment:

- Red & white blood cells and platelets
- CRP
- Serum creatinine

Further details can be found in the monograph of cyclophosphamide.

2.5 Labelling

Following information was to be added with an extra label:

"Product name"
Sponsor: UZ Gent
Contactpersoon: Prof. Dr. Fréderic Duprez, C. Heymanslaan 10, 9000 Gent, + 32 9 332 30 15
Proefpersoon identificatienummer:
Enkel voor klinische studies
Oraal gebruik

2.6 Storage conditions

Corresponding normal good practice: storage at room temperature

3. Investigational Medical Device

Not applicable.

4. Study Protocol Summary

4.1 Inclusion criteria

Histologically confirmed local, regional or combined locoregional recurrence of squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx or larynx or cancer of unknown primary (CUP) in the neck in previously irradiated tissue, with former irradiation with curative intent.

□ Patients with non-symptomatic distant metastases and local, regional or combined locoregional recurrence can be included.
□ In case of non-metastatic disease, the recurrence must be primarily unresectable recurrence and/or patients refused surgery.
□ Time interval 6-24 months after the end of the initial radio(chemo)therapy for primary head and neck cancer.
□ Decision of the Head and Neck Tumor Boards at the recruiting centre to offer salvage radio(chemo)therapy, palliative chemotherapy or anti-PD-1 antibody treatment with nivolumab for cisplatin-refractory locoregional recurrent head and neck squamous cell carcinoma..
□ Karnofsky performance status ≥ 70.
□ Age ≥ 18 years old.
□ Informed consent obtained, signed and dated before specific protocol procedures.

4.2 Exclusion criteria

Previous radiotherapy was for cT1-2 cN0 M0 glottic cancer.
□ Grade ≥ 4 late toxicity after the initial radio(chemo)therapy.
□ Brachytherapy as treatment for second primary / recurrence.
□ Previous (combination with) immunotherapy for the primary or the recurrent squamous cell carcinoma.
□ Impossibility of oral intake of cylophosphamide.
□ For patients receiving cyclophosphamide: necessary intake during therapy of allopurinol, amiodarone, digoxin, hydrochlorothiazide, indomethacin, phenobarbital, phenytoin, warfarin. clopidogrel, ticlopidine, carbamazepine, efavirenz, rifampicine, ritonavir
□ High risk for arterial blow-out: 1 of following criteria is sufficient to exclude patients:
o (1) soft tissue necrosis
o (2) skin invasion of the recurrent cancer
o (3) circumferential involvement of > 180° of a carotid artery)
□ Symptomatic distant metastases.
□ Other uncontrolled second primary tumors.
□ Pregnant or lactating women.
□ Mental condition rendering the patient unable to understand the nature, scope, and possible consequences of the study.
□ Patient unlikely to comply with protocol, i.e. uncooperative attitude, inability to return for follow-up visits, and unlikely to complete the study.

4.3 Primary endpoint

Safety, defined as absence of grade ≥ 4 acute or subacute toxicity until 3 months after treatment and absence of grade ≥ 3 mucosal ulceration or any other kind of grade ≥ 3 toxicity > 3 months after treatment).

4.4 Secondary endpoints

□ Toxicity scoring (CTCAE version …)
□ Local control
□ Overall and disease-free survival
□ QOL
□ topographic distribution of recurrence (inside/outside FDG-avid GTV)
□ time to further treatment
□ the immune response

4.5 Procedures

4.5.1. Flowchart

The outline of the study is presented in Table 2. Written informed consent will be obtained for all patients included in the study before they are registered. Patients will be identified by a unique subject number that will remain constant for the duration of the study.

Table 2. Outline of the study protocol.

Time point	SBRT	Cyclo-phospha mide (daily)	Nivo-lumab	CRF[a]	QOL	Sampling[b]	Routine blood examina tion for cyclo-phospha mide group[c]	Routine blood examina tion for nivo-lumab group[c]	PET-CT
Before start of any treatment				1, 2, 3, 5	X	IM, RN	X	X	X
Week 0			X						
Week 1	Fr 1 Fr 2	X				RN, after fr 1 RN, before fr 2			
Week 2	Fr 3[d]	X	X	4, 6		RN, before fr 3[d]	X	X	
Week 3		X		7	X		X		
Week 4		X	X				X	X	
Week 5		X					X		
Week 6		X	X	8	X	IM, RN	X	X	
Week 7		X					X		
Week 8		X	X				X	X	
Week 9									
Week 10			X	9	X		X	X	
Week 12			X					X	
Week 14			X	10	X	IM, RN		X	X
Thereafter			X[e]	11				X	

Abbreviations: CRF: case report form; fr: fraction; IM: immunomonitoring; RN: regulated necrosis; SBRT: stereotactic body radiotherapy; QOL: assessment of QOL-questionnaires

[a] All CRF's are described in chapter 11 and attached in appendix 3.

[b] Sampling: for immunomonitoring (IM), 4 EDTA tubes will be taken; for regulated necrosis (RN), 1 EDTA tube and 2 serum tubes will be taken within 1 hour after (week 1, fraction 1) or before (week 1, fraction 2 and week 2, fraction 3) radiotherapy.

[c] Routine blood examination includes erythrocytes, leucocytes and platelets, serum creatinine and CRP for the cyclophosphamide group weekly during treatment with cyclophosphamide. For the nivolumab

group, routine blood examination will be two-weekly, containing besides the above-mentioned, also liver tests, electrolytes and LDH two-weekly and monthly TSH.

[d] Only for patients receiving 3 fractions of SBRT.

[e] 2-weekly upon 1 year after treatment or disease progression

All study visits after reirradiation will take place as at the same moments as routinely done after reirradiation or palliative systemic treatment: further follow-up will be performed according to routine clinical follow-up: 1-2 weeks, 6 weeks, 10 weeks and 14 weeks (~3 months).

According to the standard scheme, patients that will receive nivolumab will be scheduled for administration of nivolumab 2-weekly upon 1 year after treatment or disease progression. Start of SBRT is 1 week after the first administration of nivolumab.

For patients treated with cyclophosphamide, start of SBRT is on the dame day of start of cyclophosphamide.

After 3 months of follow-up, there will be patient visits according to routine clinical follow-up unless toxicity or oncological evolution mandate other timepoints of follow-up: three-monthly visits during the 1st year, four-monthly visits during the 2nd year and 6-monthly visits thereafter are routinely performed until 5 years after treatment and yearly thereafter; CRF 11 will be filled in at each visit.

4.5.2.Procedures

4.5.2.1. Imaging and target definition

All patients recognized eligible and registered will undergo pre-treatment planning ^{18}F-FDG-PET/CT in treatment position that will be used for planning of dose painting by numbers. ^{18}F-FDG-PET/CT scans will be acquired as follows:

- For Ghent University Hospital: a Biograph mCT 20 Flow PET/CT scanner (Siemens, Germany) in three-dimensional mode using an axial field of view of 180 mm. Three-dimensional ^{18}F-FDG-PET scans will be acquired using a matrix of 200x200 pixels. The final voxel size is 4 x 4 x 4 mm^3.

  Contrast-enhanced CT scans of the head and neck region and thorax will be acquired using a matrix of 512 x 512 pixels at slice thickness 3 mm. ^{18}F-FDG-PET images will be reconstructed with and without attenuation correction (CT-based) using ordered subset expectation maximization.

- For Leuven University Hospital: a Siemens Hirez PET/CT scanner, in three-dimensional mode using an axial field of view of 162 mm. Three-dimensional ^{18}F-FDG-PET scans will be acquired using a matrix of 256x256 or 288x288 pixels. The final voxel size is 2 x 2 x 2 mm or better.

  Contrast-enhanced CT scans of the head and neck region and thorax will be acquired using a matrix of 512 x 512 pixels at slice thickness 2 mm. ^{18}F-FDG-PET images will be reconstructed with and without attenuation correction (CT-based) using ordered subset expectation maximization or equivalent

- For UCL-CHU Namur: a Gemini PET/CT scanner (Philips Medical Systems, Germany) in three-dimensional mode using an axial field of view of 180 mm (two bed positions, with 90 mm overlap between the two bed positions). Three-dimensional 18F-FDG-PET scans will be acquired using a matrix of 128 x 128 pixels. The final voxel size is 4 x 4 x 4 mm^3.

  Contrast-enhanced CT scans of the head and neck region and thorax will be acquired using a matrix of 512 x 512 pixels at slice thickness 3 mm. ^{18}F-FDG-PET images will be reconstructed with and without attenuation correction (CT-based) using ordered subset expectation maximization.

- The images should be acquired within a maximum period of 14 calendar days before the start of treatment.

All patients will be immobilized using a neck support and a customized thermoplastic mask extending down to the shoulders.

Imaging data will be transferred to a Raystation treatment planning system or Eclipse treatment planning system version 13 (Varian, Palo Alto, CA, USA) for CHU-UCL-Namur and UZ Leuven. Targets will be delineated on pretreatment ^{18}F-FDG-PET/CT or rigidly fused ^{18}F-FDG-PET and CT scans. The GTV will be delineated on ^{18}F-FDG-PET/CT scans using mutual information of both biological and anatomical imaging. The same window and level of ^{18}F-FDG-PET and CT scans should be used in all patients and includes a 40% cut-off of the SUV_{max} for the PET-images and for the CT with WL 350 and WW 100.

Three-dimensional expansion of the gross tumor volume (GTV) of the tumor (GTV-T) and involved nodes (GTV-N) with a 0.5 cm margin will result in the clinical target volume (CTV) adjusted to the air cavities and uninvolved bones or muscles. A margin of 2 mm will be added to the CTV to create PTV.

OARs are based on consensus guidelines [Brouwer, 2015, Appendix 2] and will include for all patients:

- spinal cord itself and the brainstem up to 5 cm cranially and caudally from the PTV.
- Parotid glands
- Mandible
- Buccal mucosa
- Lips
- Extended oral cavity
- Swallowing structures (pharyngeal constrictor muscles [superior, middle, inferior], cricopharyngeal muscle and upper oesophageal sphincter outlined together, oesophagus, and supraglottis
- Body external (rim of 5 mm within the external body contour, called "skin_wall_5mm").
- Carotid arteries

OARs outlined in some cases:

- Brachial plexus at the side of positive regional lymph nodes if located in lymph node region 3-4, according to guidelines of Hall (Appendix 3).

To obtain planning risk volumes (PRV) the spinal cord, brainstem and the brachial plexus will be expanded with a 2 mm margin.

4.5.2.2. Treatment planning, dose prescription and delivery

4.5.2.2.1. Dose prescription to target volumes

The range of dose-painting will be escalated in following levels:

- 2x 6-8Gy
- 3x 6-8Gy
- 3x 6-10Gy
- 3x 6-12Gy

The dose-painting technique limits the fraction dose in the immediate vicinity of the GTV to 6 Gy and tailors the dose inside the GTV to FDG-signal intensity. The biologically equivalent dose of 2 x 6 Gy or 3 x 6 Gy for estimating late toxicity ($\alpha/\beta = 3$) is ~22 GyE or ~33 GyE (in 2 Gy fractions) which is respectively a third or half of that of the commonly used high-dose fractionated schedules of 33-35 x 2 Gy.

The high-dose end of dose-painting (D_{high}) will be fixed at ≥ 95% of the maximum standard uptake value (SUV_{max}) of all GTV-T and GTV-N summed together, while the low-dose end of dose-painting (D_{low}) will start at 5% of SUV_{max} of all GTV-T and GTV-N summed together.

The low-end of the painted range, which is the highest dose in the surrounding normal tissues, will be fixed at 6 Gy (D_{95}). In other words, the dose prescription to the PTV around the GTV is 6 Gy (D_{95}).The high-end of the painted range will be fixed at 8, 10 or 12 Gy per fraction at the D_2. Dose fall-off outside PTV should preferably be 80% of 6 Gy per fraction at 1 cm of the PTV and 50% of 6 Gy at 2 cm of the PTV.

4.5.2.2. Dose constraints to OARs

The maximum doses for following OARs are hard constraints and should be respected in all patients, if necessary at the cost of underdosage in target volumes:

- Reirradiation > 6 and < 12 months after the end of the previous treatment:
  - fraction $D_2 \leq 2$ Gy and cumulative total summed $D_2 \leq 50$ Gy for spinal cord (PRV)
  - fraction $D_2 \leq 2$ Gy and cumulative total summed $D_2 \leq 60$ Gy for brainstem (PRV)
  - fraction D_{max} for brachial plexus (PRV) is 4.00 Gy and the volume receiving > 3.4 Gy/fraction should remain < 3 cc.
  - Fraction D_{max} for carotid arteries < 15.00 Gy and the volume receiving > 13.00 Gy/fraction should remain < 10 cc.

- Reirradiation 12-24 months after the end of the previous treatment:
  - fraction $D_2 \leq 2$ Gy for brainstem (PRV) and spinal cord (PRV) without summed dose limit.
  - fraction D_{max} for brachial plexus (PRV) is 8.00 Gy and the volume receiving > 6.8 Gy/fraction should remain < 3 cc.
  - Fraction D_{max} for carotid arteries < 15.00 Gy and the volume receiving > 13.00 Gy/fraction should remain < 10 cc.

Based on the report of AAPM Taks Group 101 following dose constraints will be applied for all patients [Benedikt et al, 2010]:

- Parts of the swallowing structures that do not overlap with GTV (pharyngeal constrictor muscles [superior, middle, inferior], cricopharyngeal muscle and upper esophageal sphincter outlined together, esophagus, and supraglottis:
  - treshold dose 5.9 Gy per fraction to < 5 cc
  - maximum point dose 8.4 Gy
- Brachial plexus:
  - treshold dose 6.8 Gy per fraction to < 3 cc
  - maximum point dose 8.0 Gy
- Skin wall 5 mm:
  - treshold dose 10.0 Gy per fraction to < 10 cc
  - maximum point dose 11.0 Gy

The doses to the other contoured OARs will depend on the localisation of the target volume and the vicinity of the OAR and will be evaluated by the treating physician. However, these doses will be recorded for study purposes.

4.5.2.3. Radiotherapy planning software

Volumetric-modulated arc therapy (VMAT) treatment planning will be based on the Ghent University Hospital's in-house developed extension of the GRATIS software package [De Gersem, 2001, De Gersem, 2001'] using a modified direct MLC-aperture optimization tool to support use of voxel-intensity values of ^{18}F-FDG-PET [Vanderstraeten, 2006; Vanderstraeten, 2006'] or Eclipse (Varian, Palo Alto, CA, USA) for CHU-UCL-Namur and UZ Leuven. Final dose computations will be done with a convolution/superposition dose engine of a Raystation treatment planning system, for Ghent University Hospital and with Analytical Anisotropic Algorithm (AAA), in Eclipse (Varian, Palo Alto, CA, USA) for CHU-UCL Namur and UZ Leuven. Treatment-planning objective of biological conformity of dose-painting-by-numbers plans assessed by the Q-factor (QF) is set to 0.05 or 5% in the GTV and GTV_{Ln}.

4.5.2.4. Radiotherapy delivery

All patients will be treated on a linear accelerator with 6 MV photon beams. Position verification using online volumetric imaging (non-planar) will be performed before each fraction.

4.5.2.5 Fractionation scheme

Based on data pointing to better locoregional control in non-consecutive delivery of SBRT with at least 48 hours between fractions of SBRT [Alite, 2016], all patients will be treated on days 1-4-7; i.e. for the first patient cohort (2 fractions) on Tuesday – Friday or on Friday – Monday and for the following cohorts on Tuesday – Friday – Monday. In case of public holidays or unforeseen breakdown of a linear accelerator or other circumstances in which the proposed fractionation scheme cannot be followed, the alternative should match the proposed protocol as much as reasonably possible, and within any case at least 48 hours between 2 fractions.

4.5.3.Collection of blood samples

Blood sampling at pre-set time points will allow to get insight and to describe the immunomodulatory effects *in vivo*. It will also allow to identify the type of cellular death being induced by the combined therapy and its correlation with therapy response. This information could then be extrapolated to other settings of combined radiotherapy with immunomodulatory medication.

Time point	Number of tubes per sampling		handling
	EDTA (9ml)	serum (6ml)	
Before therapy	5	2	S and P: 2-3 vials/tube B: 1ml vials, 7a10 milj. cells/vial
After fraction 1	1	2	S and P: 2-3 vials/tube
Before fraction 2	1	2	S and P: 2-3 vials/tube
Before fraction 3**	1	2	S and P: 2-3 vials/tube
week 6	5	2	S and P: 2-3 vials/tube B: 1ml vials, 7a10 milj. cells/vial

week 14	5	2	S and P: 2-3 vials/tube B: 1ml vials, 7a10 milj. cells/vial

If 5 EDTA tubes are collected, preservation of plasma needs to be performed in at least 1 EDTA tube.

4.5.4.Clinical evaluations

According to the outline in Table 2, clinical assessment will be performed on following time points:

- Before therapy
- At the last fraction
- Week 3
- Week 6
- Week 10
- Week 14

Toxicity will be systematically scored according to the CTCAE v. 4.0 for:

- Anemia (App. 2 page 3)
- Febrile neutropenia (App. 2 page 3)
- Fatigue (App. 2 page 22)
- Dysphagia (App. 2 page 14)
- Oral mucositis (App. 2 page 18)
- Laryngeal mucositis (App. 2 page 67)
- Pharyngeal mucositis (App. 2 page 67)
- Pharyngeal hemorrhage (App. 2 page 67)
- Pharyngeal necrosis (App. 2 page 67)
- Pharyngeal stenosis (App. 2 page 68)
- Pharyngolaryngeal pain (App. 2 page 68)
- Dry mouth (App. 2 page 13)

Quality-of-life will be assessed using the EORTC's QOL-questionnaires C30 and HN35 at following timepoints, according to Table 2:

- Before therapy
- Week 3
- Week 6
- Week 10
- Week 14

Thereafter, patients will be seen as on normal clinical follow-up: three-monthly in the first year after treatment, four-monthly in the second year of treatment and twice annually thereafter. CRF's will be filled in at each of these patient visits.

4.11 Randomisation and blinding

Not applicable, non randomised, unblinded study.

5. Study analysis

Sample size calculation:

The standard "3+3" design will be used for the this trial. To obtain more precise toxicity rate of the MTD we will double the number of patients at the first dose prescription that gives totally 6 patients. The 3 remaining dose levels will include 3 patients each. Thus, fifteen (6+3+3+3) patients will be included in this radiotherapy dose finding study investigating the MTD.

The number of patients will be doubled in case of 2 DLTs at the dose prescription I and 1 DLT at dose prescriptions II-IV with DLT in a maximum of 10 out of 30 patients.

The minimal number of patients recruited in the trial will thus be: 4 (in case of 4 consecutive DLT's in the first 4 patients)

The maximal number of patients recruited in the trial will thus be: 30 (in case of doubling all 4 levels of escalation: [(6 X 2) + (3 X 2) + (3 X 2) + (3 X 2)] = 30).

Analysis of the samples

No analysis of the samples have been performed up to now.

Statistical analysis

With the standard 3+3 design of this radiotherapy dose finding study, only descriptive statistics will be used. All statistics will be performed under supervision of the principal investigator of the study.

6. Independent Ethics Committee and Competent Authority

The study has been approved by the central ethical commission at the sponsors' site (UZ Ghent) and by the ethical commissions of the 2 other participating centers (UZ Leuven and CHU/UCL Namur).

Schematical overview:

OVERVIEW APPROVED DOCUMENTS		
Initial submission: - Protocol version 4.0 dd 01/06/2017 - ICF version 3.0 dd 27/07/2017 – Dutch and French version - Patient questionnaires dd. EORTC QLQ-H&N (N&F) and EORTC QLQ-C30 (N&F)	**Approval date Central EC:** 31/07/2017	**Approval date FAMPH:** 29/05/2017
Amendment 1: - Addition of CHU/UCL as participating centre. - Note: this amendment was necessary although the initial submission included this centre. Due to an administrative mistake in CHU/UCL Namur the	**Approval date Central EC:** 30/08/2017	**Approval date FAMPH:** N.A., no substantial amendment

submission was not completely fulfilled in that centre. There were no other changes then this administrative change.		
Amendment 2: - Protocol version 5.0, dd. 08/03/2018 - ICF version 4.0 dd 28/03/2018 – Dutch and French version - Patients questionnaires EORTC QLQ-H&N (N&F) and EORTC-QLQ-C30 (N&F) - Subject: **substantial amendment**: include nivolumab as immunomodulating agent.	**Approval date Central EC:** 01/08/2018	**Approval date FAMPH:** 30/07/2018
Amendment 2: - Protocol version 6.0, dd. 08/04/2020 - ICF version 4.1 dd 28/03/2018 – Dutch and French version - Subject: - change of PI to Prof. F. Duprez after retirement of initial PI, Prof. W. De Neve in UZ Ghent and change of PI to Dr. Deheneffe after departure of Dr. Daisne in CHU/UCL Namur	**Approval date Central EC:** 11/05/2020	**Approval date FAMPH:** N.A., no substantial amendment

7. Results

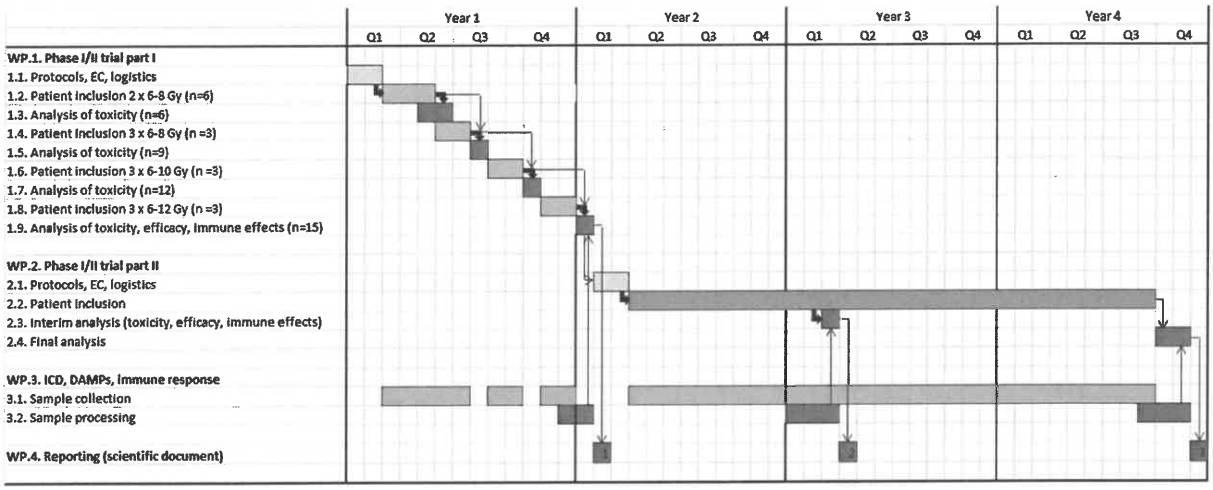
7.1 Subject enrollment and demographics

Recruitment for the study started in August 2017, with 2 patients signing the ICF in a short period of time, and with 1 patient being excluded from the study after objectification of progressive disease. Thereby, initially 1 patient started with the study treatment. Shortly afterwards, there was a decline in recruitment that we attributed to the inntroduction of the reimbursement for nivolumab in Belgium, that could be administrated in an identical indication where there had never been a valuable treatment option.

In an effort to restore patient recruitment, the study inclusion criteria were adjusted to reflect the new reimbursement criteria for nivolumab, allowing for inclusion with standard-of-care nivolumab. This amendment has been adopted in August 2018. Since then, 4 more patients have been recruited at UZ Gent, again in 1 patient where no treatment was possible after objectification of progressive disease, 1 patient in UZ Leuven and 1 patient in CHU / UCL Namur. In UZ Gent, a total of 2 of the 6 recruited patients were diagnosed with rapidly progressive disease between signs of ICF and the start of study therapy, so that they could no longer be enrolled; after all, both patients meanwhile showed one of the exclusion criteria of the study, i.e. tumor circumferential around the carotid artery > 180 °. So to date a total of 7 patients have been recruited and 6 patients have also been treated effectively

Due to the new reimbursement criteria for nivolumab (just prior to the start of the inclusion in September 2017), and more recently pembrolizumab and other competing trials (e.g. EORTC Upstream, several company-driven phase I trials), we find that patients from other centers not be referred for the current study. In our own centers, too, priority is regularly given to other treatments / trials after multidisciplinary discussions. Patients who cannot be included in these trials often have unfavorable prognostic characteristics that also prevent inclusion in this trial. It also seemed that, when proposing the trial to potential patients, inclusion was regularly declined. In those patients accepting inclusion in the trial, we were confronted with progressive disease in 2/8 cases, as a result of which exclusion criteria were met during the screening period.

For documentation find below the initial planned time course:



Due to the circumstances described above, we did not went further then WP.1. – 1.3 and WP.3. 3.1.

The numbers of subjects included per center are as follows:

Site	Not active yet?	Active?	Closed?	Number of Subjects included?	Date of first inclusion?	Last patient – last visit
UZ Gent	☐	☐	☒	4	19-Sep-2017	19-Sep-2019
UZ Leuven	☐	☐	☒	1	03-Oct-2018	24-Jul-2019
CHU Namur	☐	☐	☒	1	13-Nov-2017	24-Nov-2017

The numbers of subjects exposed per immunomodulating agent are as follows:

Treatment	Dose	Number of subjects
Cyclophosphamide (Investigational Drug)	50 mg, QOD	4
Nivolumab	SOC	2

The available demographic data are shown below:

Age range	No of female subjects	No of male subjects	Total No of subjects
53-75	0	6	6

At this time in the trial 6 subjects were included. The current accrual rate does not resemble the planned accrual rate due to much less indications as anticipated at the start of the trial. It is probable that the reimbursement of nivolumab, installed after starting the trial, has contributed to the absence of referrals from non-participating centers. Previously it was not possible to offer these patients a relevant treatment option, whereas currently this is the case.

Moreover, since more competing studies are launched for this patient population and since other molecules for immunotherapy in this indication are meanwhile approved and reimbursed, it is very likely that the primary endpoint will ever be reached. Therefore, all 3 collaborating principal investigators in the 3 centers unanimously decided to stop the trial.

7.2 Study specific results

No serious adverse events have been observed in the first dose cohort (see above). All but 1 patient meanwhile deceased:

- Pneumonia (non-SUSAR): n = 1.
- Progressive disease: n = 4.

No further analysis has been performed.

8. Safety

All serious adverse events during the reporting period are summarized in the table below:

SAE Overview				
Subject ID	Study Arm (if applicable)	SUSAR (Y/N)	SAE Description	Outcome (ongoing, resolved, death, …)
R1-002	N/A	N	Pain	Resolved
R2-001	N/A	Y	Auto-immune pneumonitis	Ongoing at moment of death due to other cause.

All SAE's were reported within the timelines as mentioned in the latest approved protocol to the sponsor.

Cumulative Summary Tabulations of Serious Adverse Events

System Organ Class	Total up to 3 DEC 2021
Gastro-intestinal disorder	1
General disorder	1
Respiratory, thoracic and mediastinal disorders	1

9. Device deficiencies

Not applicable.

10. Protocol deviations

There are no protocol deviations to be reported.

11. Discussion and overall conclusions

As has been described above, it was very unlikely that the primary endpoint would ever be reached given (1) the very slow recruitment until now, (2) new upcoming competing trials and (3) reimbursement for novel molecules that could be administrated in routine clinical treatments rendering the option of study treatment less interesting.

Therefore, all 3 collaborating principal investigators in the 3 centers unanimously decided on 03 December 2020 to stop the trial.

No overall conclusions can be made with the current data, except for the safety of the first and complete investigated radiotherapy dose treshold of 2 fractions of 6-8 Gy using dose painting by numbers with immunomodulating nivolumab or cyclophosphamide.

12. References

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