Nitroglycerin in NSCLC.

Nitroglycerin as a sensitizer in the treatment of non small cell lung cancer: a phase II trial.

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1 Synopsis and flowchart

1.1. Synopsis.

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Study
Single centre non-randomized combination phase II trial.

Rationale

Tumor hypoxia is a well known factor negatively influencing the response of numerous types of cancer to chemotherapy or radiotherapy. Tumor hypoxia is due to many factors, which
can be patient-related (e.g. anaemia or vascular insufficiency), but also tumor-related (e.g. abnormal tumor vasculature).

The primary physiological function of the tumor vasculature is to support perfusion, the nutritive flow of blood through the tissues. Vascular physiology can be studied non-invasively in human subjects using imaging methods such as positron emission tomography (PET), magnetic resonance imaging (MRI), X-ray computed tomography (CT), and Doppler ultrasound (DU). (1-4) Tumor perfusion has a prognostic value but is also a key process to allow drug penetration in tumor tissues.

Nitroglycerin is a nitric oxide donor which is mainly known as a vasodilating agent used in ischemic heart disease. It has also been shown to increase tumor blood flow in animal and human tumors.

The addition of nitroglycerin to chemotherapy in non small cell lung cancer has been shown to generate very favorable response rates with respect to standard treatment schedules(5). Theoretically nitroglycerin might reduce resistance to chemotherapy via a plethora of different effects: better tumor perfusion, direct effects of NO on cancer cells, increase in activated p53 protein and via an increased blood flow in the tumor with as consequence a higher drug concentration in the tumor (6).

In mice, NO donors such as isosorbide dinitrate have been shown to decrease tumor hypoxia by better tumor perfusion, which could enhance radiotherapy responses (7).

| Table 1. Dose-dependent effect of isosorbide dinitrate in comparison with carbogen on pO₂ in TLT tumors |
|---------------------------------------------------------------|
| Treatment |         pO₂ (± standard error) | pO₂ (± standard error) | Responsive tumors% |
|           | before treatment (mmHg) | 30 min after treatment (mmHg) |                             |
| Isosorbide dinitrate |                         |                            |                             |
| 0.64 mg/kg  | 1.29 ± 0.25 | 2.26 ± 0.25 | 0% (14)                         |
| 0.1 mg/kg   | 1.91 ± 0.22 | 4.78 ± 0.36 | 24% (12)                        |
| 0.2 mg/kg   | 2.05 ± 0.37 | 6.95 ± 0.83 | 79% (14)                        |
| Carbogen    |                         |                            |                             |
| 4 L/min     | 1.01 ± 0.32 | 24.17 ± 5.06 | 100% (10)                       |
| Control     |                         | 2.82 ± 0.29 | 0% (10)                          |
| 0.9% NaCl   |                         |                            |                             |

*Significant difference before and after treatments (t-test): t < 0.05, t < 0.01. A tumor is considered responsive when pO₂ is elevated by an arbitrary additional 3 mmHg after a 30-min treatment.
The promising results of the combination of nitroglycerin with chemotherapy and the theoretical and preclinical basis for a hypoxia reducing and radiosensitizing effect on cancer cells, make this an interesting compound to investigate in a phase II trial.

Primary endpoint of this trial will be to demonstrate a survival benefit of the addition of nitroglycerin to radiotherapy for NSCLC.

Translational research will be a part of this trial: by performing upfront measurements of perfusion and hypoxia and quantifying the effect of nitroglycerin on these measurements we hope to clarify the mechanism of action of nitroglycerin in NSCLC.

During this trial, acute toxicity will be monitored closely during and after radiotherapy.

Study Centres
Maastricht Radiation Oncology (MAASTRO Clinic)
University Hospital Maastricht

Planned period

2011-2015

Primary objective

Demonstrate an increase of 2-year overall survival (OS) of 15 % (from 50% to 65 %) vs historical controls of the addition of nitroglycerin to radiotherapy (±chemotherapy) of stage I-IV NSCLC.
Secondary objectives

1. To demonstrate the effect of a nitroglycerin patch on enhancement of tumor perfusion.
2. To demonstrate the effect of a nitroglycerin patch on enhancement of tumor oxygenation.
3. Toxicity (CTC AE 4.0).
4. To evaluate the possible prognostic value of perfusion CT and hypoxia scans on treatment with nitroglycerin of NSCLC
5. Evaluate response on an 18-FDG PET-CT scan 2.5 months after the end of treatment and correlate these findings with pre-radiotherapy FDG/ hypoxia PET-scans and perfusion CT scans.

Interventions

Patients will receive a standard low dose nitroglycerin patch, for 12 hours daily during the whole course of radiotherapy.

Patients will be required to come to the hospital for approximately 1 day on scanning days.

"Baseline" scans: a DCE-CT scan is performed, followed by an injection of HX-4 in the early afternoon and 1 HX-4 scan at 240 minutes post-injection.

"Nitroglycerin" scans: 2 days later the same scans are repeated at a minimum of 3 hours after application of a standard dose nitroglycerin patch.

Remark: All patients will receive the baseline scans, without nitroglycerin.

After the inclusion of 40 patients completing baseline and nitroglycerin scans, the nitroglycerin scans will be abandoned and subsequently included patients will only receive the baseline scans.

Nature of burden and risks
The extra burden in this trial consists of

- nitroglycerin patch from day 1 of radiotherapy to the last day of radiotherapy

2 DCE CT scans (1 with and 1 without nitroglycerin) AND 2 HX-4 PET-scans ((1 with and 1 without nitroglycerin) in 40 patients.
1 DCE CT scan and 1 HX-4 PET scan without nitroglycerin in 20 patients.

Risks: DCE-CT scans and HX-4 PET-CT-scans will add on average 7-7.5 and 20-25 mSV respectively to the radiation dose received (8)
When compared to the 65000 mGy which is on average administered to patients treated with radiotherapy for NSCLC at Maastro Clinic, this added radiation dose by the extra scans is negligible.

Contrast enhancement has the known risks of allergy and renal failure, which is generally a low risk. To minimize the risk of renal impairment by a bolus injection of contrast used in DCE-CT scanning, the DCE-CT scans are performed with a minimum of 48 hours apart and patients are encouraged to augment their fluid intake between scans.

Nitroglycerin has as most common side effects: headache (60% of patients), light-headedness (6% of patients), syncope (4% of patients).

The combination of nitroglycerin with chemotherapy has been proven to be safe in a large phase II trial and reports of toxicity of the combination of nitroglycerin and radiotherapy have never been made. However, theoretically the perfusion of normal tissue might also be influenced, leading to enhanced radiosensitivity of normal tissue.

Therefore patients will be closely monitored for enhanced side effects during and after radiotherapy. A stopping rule has been foreseen in case of excess toxicity.

Number of patients
60
Inclusion criteria

- Non-small cell lung cancer stage IB-IV amenable for radiotherapy with curative intent.
- (Stage IV patients with oligometastatic (1-4 metastases) NSCLC are regularly treated radically in the IKL region)
- Patients not included in the PET-boost trial or the Lucanix trial
- WHO performance status 0-2
- Willing and able to comply with the study prescriptions
- 18 years or older
- Ability to give and having given written informed consent before patient registration
- No recent (< 3 months) severe cardiac disease (NYHA class >1) (congestive heart failure, infarction)
- No radiotherapy in 4 weeks prior to this study
- No treatment with investigational drugs in 4 weeks prior to or during this study.
- No known allergy to nitroglycerin or nitroglycerin patch.
- No known allergy to iodine based contrast agents
- No use of vaso-dilators (calcium channel blockers, nitrates or 5-fosfodiesterase inhibitors)
- No symptomatic hypotension
- No other active malignancy
- No major surgery (excluding diagnostic procedures like e.g. mediastinoscopy) in previous 4 weeks
- Adequate renal function: calculated creatinine clearance at least 60 ml/min
1.2. Flowchart

Main Phase II trial: 60 patients NSCLC stage Ib-IV

Initial work-up:
CTC AE 4.0
Blood sample
FDG-PET-CT

Transderm Nitro 5 mg + radiotherapy +/- chemotherapy

Primary endpoint: Overall survival at 2 years
Secondary endpoint: Toxicity (CTC AE 4.0)

Translational research:

60 patients baseline HX-4 PET + DCE-CT

40 patients Nitroglycerin + HX-4 PET + DCE-CT

Endpoints:
- 60 patients: prognostic impact on survival of hypoxia/ perfusion
- 40 patients:
  - quantification of effect of nitroglycerin on tumour perfusion
  - quantification of effect of nitroglycerin on tumour hypoxia
1.3. **Rationale for the study.**

1.3.1. **NO-donors in anti-cancer therapy: evidence.**

Nitric oxide is a ubiquitous molecule which has numerous functions in tumoral cellular processes.

There is a tremendous body of scientific research on this molecule in the past decade, resulting in a couple of interesting phase II trials which demonstrate the regulatory effect of NO-donors on cancer cells.

In a randomized phase 2 trial by Yasuda et al nitroglycerin has been successfully combined at a dose of 25 mg daily for 5 days each chemo cycle with cisplatin and vinorelbine in non small cell lung cancer, enhancing chemotherapy response (5). The toxicity profile between the 2 arms was not significantly different.

In a non-randomized phase 2 trial by Siemens et al delivered a very low dose of nitroglycerin (Minitran 5 patch (18 mg) cut in 6 pieces, delivering 0.033 mg/h in stead of 0.2 mg/h normally) to patients with biochemical recurrence (PSA-failure) after primary therapy. After 24 months the PSA doubling time was more than 31 months versus 12.8 months before the start of therapy, demonstrating the inhibitory effect of nitroglycerin on prostate cancer cells. (9)

Currently a Mexican phase 2 trial is recruiting 40 patients to evaluate the efficacy of nitroglycerin added to concurrent chemoradiotherapy in NSCLC stage III. (Clinical trials.gov identifier NCT00886405).

1.3.2. **Mechanism of Action**

Nitroglycerin is a substance known for more than a century, not only because of it´ s original use as an explosive, but in medicine it is used mainly for it´ s vasodilating properties. Nitroglycerine has been safely used for decades in patients with angor pectoris in various forms: as a spray, a sublingual tablet or a transdermal patch.
Nitroglycerin is a nitric oxide (NO) donor. Nitroglycerin is converted intracellularly by mitochondrial aldehyde dehydrogenase (mtADH) into 1,2-glyceryldinitrate and NO via an S-nitrosothiol or nitrite intermediate. (10) The produced nitric oxide then exerts a plethora of effects which can be exploited in medicine.

As a vasodilator: NO is released in smooth muscle and activates guanylyl cyclase, thereby increasing cGMP concentration and resulting in smooth muscle relaxation. Preclinical data show that nitric oxide is able to enhance tumor perfusion in animals. Jordan et al evaluated the effect of isosorbide dinitrate and carbogen (as a reference) on tumor perfusion in liver tumors implanted in mouse thighs. These were imaged using magnetic resonance imaging (MRI) and EPR oximetry (1.1 GHz) on 52 mice. A significant increase in MRI intensity was observed for both treatments in comparison with the control group. EPR oximetry showed a dose-dependent increase in tumor pO2 for isosorbide dinitrate.(7)

Not only is there an increase in tumor bloodflow, also direct effects on stabilization of p53 and degradation of Hif-1 alpha have been found(6). Decreased hypoxic biomarkers (eg VEGF, P-glycoprotein) have been found in patients with NSCLC treated with nitroglycerin patches for 3 days prior to surgery when compared to non-treated individuals (11). There might also be a supplementary effect on the MHC-molecules, rendering tumor cells more `visible` to the immune system (12).

Furthermore, hypoxia has been shown in vitro to increase the invasiveness of cancer cells in an NO-mediated manner, which can be blocked by NO-donors.(13) Also, NO has been shown to block the production of TGF-beta in tumor cells. Since TGF beta is also proposed as one of the key factors in mediating the development of late fibrosis in response to injury and inhibition of TGF beta has been shown in mice to prevent radiation induced lung damage, nitric oxide might be interesting in diminishing late radiation damage.(14)

Due to these effects and the effect on tumor bloodflow and decrease of hypoxia nitroglycerin might also be interesting as a radio- and chemosensitizing agent, both locally (decreasing hypoxia) as well as systemically (enhancing the antitumoral immune response).
The principals of chemosensitization and radiosensitization of tumor cells have been demonstrated in vitro and in animals by several groups:

Konovalova showed a significant increase in life span and number of survivors among mice bearing leukemias P388 and L-1210 treated with cisplatin or doxorubicin when these treatments were combined with an NO donor. (15)

Riganti et al used a known doxorubicin resistant colon cancer cell line (HT29-dx) to test the effects of an NO donor on the efficacy of doxorubicin: the NO donor SNAP reversed the resistance of doxorubicine by inhibiting the efflux of the drug. Similar experiments have also been performed and proved the chemosensitization of melphalan and fludarabine in breast cancer cells and leukemia cells to melphalan and fludarabine respectively. (16)

In humans indirect evidence for a chemosensitizing effect of nitric oxide has also been provided by Choi et al, who correlated the presence of genes encoding for nitric oxide synthase to survival of breast cancer patients in a Southwest Oncology Group trial on adjuvant chemotherapy. Women receiving chemotherapy and in whom genes coding for low nitric oxide production were found had a 2-fold increase in hazard of progression (hazard ratio, 2.32; 95% confidence interval, 1.26-4.25). (17)

Radiosensitization has also been reported in human tumor cells: in 1996 Verovski et al already showed in human pancreatic cancer cell lines selected for high radioresistance that addition of NO through the nitric oxide donor sodium nitroprusside increased radiosensitivity with an enhancement ratio of 1.9. (18)

Griffin and colleagues reported comparable activity for NO donors DEA/NO and spermine nonoate (SPER/NO), with enhancement ratios of 2.8–3.0 in hypoxic SCK mammary carcinoma cultures. No radiosensitization was found in non-hypoxic cells, which might be an advantage in the context of radiotherapy, with tumors tending to be more hypoxic than the surrounding normal tissue. (19)

Wardman et al even suggested a more potent radiosensitization by NO than oxygen in anoxic V79 lung fibroblast cells, with marked radiosensitization still observed at a low concentration of 40 ppm NO in suspension, while no radiosensitization was noted anymore
at a concentration of 50 ppm oxygen. In fact comparison with O2 showed that 1000 ppm O2 appeared no more effective than 80 ppm NO, making nitric oxide a far more potent radiosensitizer than oxygen. (20)

The rationale for providing exogenous NO rather than stimulating the inducible nitric oxide synthase activity in hypoxic tumors was provided by an experiment of Singh et al, in which they showed that no radiosensitization was observed when iNOS (inducible Nitric Oxide Synthase) was stimulated by cytokines under low-oxygen conditions (<0.01% oxygen). The endogenous NO production by iNOS was not augmented exactly because oxygen is necessary for the production of endogenous nitric oxide by cells. Therefore substitution of NO by exogenous NO donors is necessary to provide a radiosensitizing effect to hypoxic tumours. (21)

Although there haven`t been any phase I trials on combining radiotherapy and chemotherapy with NO donors, no reports have been made on excess radiotoxicity in the large number of patients using these medications (Search on Pubmed on 24-2-2011 using key-words “radiotherapy” “nitroglycerin” “toxicity”). Furthermore: the reported phase II trial of Yasuda showed no excess toxicity when combining nitroglycerin to chemotherapy.

The primary aim of the present study is to demonstrate a survival benefit of adding nitroglycerin to standard treatment for NSCLC stages I-IV. Secondary endpoints aim at clarifying the mechanisms underlying this effect and identifying subgroups of patients more likely to benefit from the addition of nitroglycerin to therapy. Since there are no data on the toxicity of nitroglycerin combined with radiotherapy, as a secondary endpoint toxicity will be monitored closely during and after therapy.
1.4. Translational research:

1.4.1. Translational research in this trial is focused on elucidating the mechanism of action of nitroglycerin by demonstrating
1. the presence (or absence) of a perfusion effect of nitroglycerin in NSCLC
2. the translation of a perfusion effect of nitroglycerin into a decrease of hypoxia
3. the prognostic value on overall survival of different pre-treatment levels of perfusion and/or hypoxia in NSCLC

**Hypothesis:**
The effect of nitroglycerin on perfusion will be most marked in the 25% of patients with the lowest pre-treatment values of perfusion, described by the permeability (in ml/100ml/min). Permeability is chosen because this is the parameter with the smallest confidence interval on test-retest values in the series described by the Mount Vernon group. (3)

**Set-up:**
Patients enrolled in the trial will be requested to undergo repeat scanning with perfusion CT (DCE-CT) scans and hypoxia PET-scans at 2 separate occasions before treatment start;
"Baseline" scans: 1 DCE-CT and 1 HX-4-PET-scan
"Nitroglycerin" scans: 1 DCE-CT and 1 HX-4 PET-scan after administration of nitroglycerin

After the first 40 patients completing all scans, the subsequently included 20 patients will only be requested to undergo the baseline scans to evaluate the prognostic value of DCE-CT and hypoxia scanning.

The number of 40 patients is chosen to have a sample size which gives a flavour of the normal Gaussian distribution in our patient population.

Assuming this Gaussian distribution in this series of patients, 3 groups of patients will thus be identified:
1. The 10 patients with the lowest permeability values will represent the quartile with "low perfusion".
2. the 10 patients with the highest permeability values will represent the quartile with “low perfusion”.
3. the 20 other patients will represent the “middle perfusion” group.

Effect of nitroglycerin on perfusion (permeability and whole tumour blood volume) and hypoxia (tumour-to-blood ratio (TBR)) can then be compared between the different groups.

1.4.2. DCE-CT scanning

Dynamic contrast enhanced computed tomography (DCE-CT), also known as perfusion CT, is a non-invasive, validated method for studying the tumour vasculature. Different vascular parameters including perfusion, blood volume and extraction fraction can be assessed using physiologically based mathematical algorithms, for instance, Patlak based analysis, which has been validated previously in an animal study. Conventionally, DCE-CT has been performed as an axial technique at a single anatomical level, thus z-axis coverage has been limited, and dependent on detector configuration. However the tumour vasculature is both structurally and functionally heterogeneous and it is likely that changes seen within a small tumour volume will be misleading.

The development of helical volumetric DCE-CT, with tissue volume coverage up to 28cm on the z-axis with state-of-the art CT provides a more representative depiction of the spatial heterogeneity of tumour vascularity, reduces error from positional artefacts, and is more reproducible than conventional single-tumour-level techniques particularly at sites susceptible to motion, thus has the potential to evaluate changes in tumour vasculature with greater accuracy than previous single level techniques.

In an article investigating the reproducibility of perfusion measurements using DCE-CT whole tumour blood volume measurements proved to be far more reproducible than single slice measurements. (8) (Table taken from Ng et al)
Therefore we will use the technique described by Ng et al to measure whole tumour blood volume and permeability to quantify tumour perfusion.

1.4.3. HX-4 PET

1.4.4. Theoretical background for the use of HX-4 PET

The 2-nitroimidazoles are the most widely used compounds for imaging of hypoxia. Their hypoxia-specific uptake has mainly been validated in easily accessible tumors such as head and neck cancer.

$[\text{F}^{18}]\text{Fluoromisonidazole ($[\text{F}^{18}]\text{MISO}$)}$ is the most widely tested and best known compound of the 2-nitroimidazole derivatives proposed for hypoxia imaging with PET. However, $[\text{F}^{18}]\text{MISO}$ displays a relatively low uptake in hypoxic lesions with a tumor to muscle ratio of 1.7-1.9 (4, 22) and median SUVmax values around of 2-2.5 (4, 23) Also a considerable amount of metabolites are produced when using this compound. Furthermore, the relatively slow clearance from normoxic tissue is a limiting factor for the clinical usefulness of this compound, resulting in a scarce use in only a limited number of centres across the world.

The 2-nitroimidazole nucleoside analogue, $3-[\text{F}^{18}]\text{fluoro-}2-(4-((2\text{-nitro}-1\text{-H-imidazol}-1\text{-yl})\text{methyl}-1\text{H}-1,2,3\text{-triazol}-1\text{-yl})\text{propan}-1\text{-ol ($[\text{F}^{18}]\text{HX4}$)}}$ was developed to achieve better water solubility and faster clearance than most known nitro-imidazoles and is therefore expected to have better pharmacokinetic properties.
Animal experiments and human data

Animal experiments were performed in rats and mice by Ludwig Dubois at Maastro describing the hypoxia-dependent uptake of $^{18}$F HX-4 in tumor models and comparing quantitatively the tumor to blood ratios of $^{18}$F HX-4, $^{18}$F-EF3, $^{18}$F Miso and $^{18}$F FAZA.

Using serial PET-CT scanning after injection of $^{18}$F HX-4, a clear accumulation of HX-4 with time was observed. During the first hour post injection (pi), the activity in muscle and tumor was comparable, from 2 hours pi the tumor to blood ratio (TBR) started to increase, resulting in a mean TBR of 2.5 ± 0.5 at 4 hours pi. No further accumulation was seen after 4 hours. The maximum TBR was 7.6±3.8 at 4 hours pi. Clearance from muscle also occurred during this time, resulting in significantly lower muscle-to-blood ratios (MBR) after 4 hours.

$^{18}$F Miso and $^{18}$F EF3 are known to have similar fast and uniform distributions in normal and tumor tissues. (24) When compared to $^{18}$F EF3, $^{18}$F HX-4 showed similar mean TBR, but a significantly higher maximal TBR. MBR was equal for 2 hours pi, but from 2 hours pi to 4 hours pi, MBR decreased for $^{18}$F HX-4, while remaining constant for $^{18}$F EF3, indicating faster clearance from normal tissue for $^{18}$F HX-4.

A test of hypoxia-dependency of $^{18}$F HX-4 uptake was also performed: During carbogen-nicotinamide breathing (enhancing tumor oxygenation), maximum $^{18}$F HX-4 uptake in the tumor was found to be on average 40% lower than baseline (during normal air breathing). This difference was not found in normal tissue. When animals were breathing a hypoxic mixture of 7% oxygen however a significant increase of the maximum $^{18}$F HX-4 uptake with 20 % was observed, which again was not shown for normal tissue.

Comparing the uptake of $^{18}$F HX-4, $^{18}$F Miso and $^{18}$F FAZA in human xenografts of different types of cancer, no significant differences in tumor uptake were observed, but $^{18}$F HX-4 had a significantly faster clearance from normal tissue than the other tracers.
A Phase 0 trial was performed by Doss et al in 3 rhesus monkeys and 4 humans determining the biodistribution of $[^{18}\text{F}]\text{HX4}$ and estimating the radiation exposure of PET-scans using $[^{18}\text{F}]\text{HX4}$. Injected dose in humans was 422±142MBq (range 240–636 MBq) of $^{18}\text{F}-\text{HX4}$. Consequently, 5 scans were taken at 15, 80, 120, 150, 180 min after injection, vital signs were monitored and blood and urine samples were taken before each PET scan. Three male rhesus monkeys (age 6–8 years, 10–11 kg) were also injected with an IV bolus of 189±3MBq of $^{18}\text{F}-\text{HX4}$, after which 24 whole body PET scans were acquired during 200 minutes. Vital signs were monitored and blood samples were taken before each PET scan.

The injection of 422±142MBq of $^{18}\text{F}-\text{HX4}$ in humans and monkeys produced no clinically significant effects on vital signs (blood pressure, temperature, pulse, and ECG) and blood tests during the first 3–4 h observation period after administration and the follow-up visit at 24 h. The analysis of plasma and urine samples of three participants showed that the concentration of unmetabolized $^{18}\text{F}-\text{HX4}$ decreased slowly from 94%-99% at 5 min to 82%-84% at 120 min after the $^{18}\text{F}-\text{HX4}$ injection. Thus, $^{18}\text{F}-\text{HX4}$ remains intact in the blood during an entire HX4-PET/CT study. (25)

Residence times of HX-4 in the body in humans were as follows (Table 1, taken from Doss et al)
The radioactivity of HX-4 is mainly excreted renally, which makes the bladder the organ with the highest exposure. 45% of the injected activity is voided 3.6 hours after injection. A comparable pattern was found in the rhesus monkeys, with the bladder being the organ with the highest exposure.

The total body absorbed dose was compared to different hypoxia and PET-tracers (Table 6, taken from Doss et al (25))

| Table 1 | Residence times of source organs for participants injected with $^{18}$F-HX4 ($n=4$, mean ± SD) |
|---------|------------------------------------------------------------------------------------------|
| Organ   | Residence time (h)                                                                        |
| Brain   | 0.022 ± 0.009                                                                            |
| Gallbladder | 0.0088 ± 0.0045                                                                          |
| Kidneys | 0.024 ± 0.004                                                                            |
| Liver   | 0.038 ± 0.023                                                                            |
| Distal colon | 0.018 ± 0.004                                                                            |
| Small intestine | 0.022 ± 0.004                                                                            |
| Testes  | 0.0010 ± 0.0003                                                                          |
| Proximal colon | 0.016 ± 0.007                                                                            |
| Bladder (1-h void) | 0.163 ± 0.021                                                                            |
| Bladder (4.8-h void) | 0.615 ± 0.082                                                                            |
| Remainder of body | 1.52 ± 0.15                                                                 |

Table 6 Comparison of dose between $^{18}$F-HX4 and other $^{18}$F-based imaging agents [9,18,19]

|             | HX4 1 h void | HX4 4.8 h void | FDG 4.8 h void | FMISO 4 h void | FETNIM 2 h void | FETNIM 4 h void |
|-------------|--------------|----------------|---------------|----------------|----------------|----------------|
| Total body dose (µGy/MBq) | 8             | 10             | 11            | 13             | 11             | 11             |
| Effective Dose (µSv/MBq)     | 14            | 27             | 19            | –              | 15             | 19             |
The total body dose of HX-4 is thus comparable to other commonly used PET-tracers and due to the fact the main part of the effective dose is due to the residence of HX-4 in the bladder until voiding, unlike the other hypoxic tracers which have longer residence time in normal tissue (eg muscle) the effective dose can be reduced considerably by adequate hydration prior to injection and frequent voiding, making the tracer a favorable alternative to other hypoxia tracers.

Due to the relatively short physical half-life of $^{18}$F (110 min) and biological half-life of HX4 (less than 3 h), after 1 day the radioactivity of both the drug and its metabolites have disappeared and no additional toxicity from the radiation is expected.

In a phase I trial the safety of [18F]HX4 was examined in six patients with stage IV cancer of various histologies (4/6 NSCLC, 1/6 thymus carcinoma, 1/6 colorectal carcinoma). In the first 3 patients a single dose of up to 222 MBq [18F]HX4 was injected while in a second step a single dose of up to 444 MBq was used in 3 patients. Toxicity was scored on day 0 and on days 3 and 7 after injection, according to the CTCAE (Common Terminology Criteria for Adverse Events) version 3.0 scoring system. No toxicity was observed according to the CTC AE 3.0 criteria.

The scanning time was unfortunately limited to 120 minutes post-injection to decrease the burden for the patients and dynamic scanning was omitted, so no correction could be made for heterogeneity in tumor perfusion when calculating the TMR. (26)

One clinical phase II trial with [18F]HX4 has been undertaken (unpublished data, obtained at investigators meeting 3-2011): HX-4 was shown to have an intra-patient (test-retest) variability in head and neck and lung cancer patients of 5.2 %. The intra-tumoral distribution showed a good match in all evaluated patients.
## HX-4 Phase 2a, preliminary results

Test/Repeat Test within 48 hours in Lung, H&N, Cervix and Rectal Cancer Patients, N=34 (excl. 1 Outlier)

| Sub-population                                           | Mean Difference of HX4 T/B Ratios | Correlation |
|----------------------------------------------------------|----------------------------------|-------------|
| For the entire study population (N=34, excl. 1 outlier)  | 6.30%                            | 0.96        |
| For the H&N and Lung cancer sub-population (N=22)        | 5.20%                            | 0.98        |
| For the Cervix and Rectal cancer population (N=12)       | 11.5% C, 8.2% R                  | 0.48 C, 0.41 R |

Reproducibility of intra-tumoral tracer distribution:

- Lung and H&N cancer, pre-treatment reproducib.: In 6/6 cases good match
- Lung and H&N cancer, pre to post treatment reproducib.: Reduced uptake, but overall similar distribution
2 Detailed information

2.1. Study objectives

2.1.1. Primary endpoints

- Demonstrate an absolute increase in 2 year overall survival of 15 % vs historical controls

2.1.2. Secondary endpoints

- Decrease of hypoxia (less uptake of HX-4 in the tumor) on PET-scan described by TBR
- Tumor perfusion (of the largest lesion tumor/ node) on DCECT-scan described by Whole tumour Blood Volume and Tumour Permeability
- Evaluating prognostic effect of perfusion and hypoxia values in patients treated with nitroglycerin.
- Acute toxicity (CTC AE 4.0)
- Evaluate response on an 18-FDG PET-CT scan 2.5 months (70 days ± 10 days) after the end of treatment and correlate these findings with pre-radiotherapy FDG/ hypoxia PET-scans and perfusion CT scans.

2.2. Trial design

Single centre non-randomized phase II combination trial.

2.3. Study duration and termination

The expected duration of enrollment will be 48 months.
The expected start date is december 2011.
The enrolment finished date is expected to be december 2015.
The study will be terminated when the primary endpoint has been reached.

Study discontinuation for the individual patient. 
Based on the decision of either the principal treating physician or the patient himself, the patient can discontinue participation at any time. Reasons for this decision (medical reasons, personal decision) should be documented and communicated with the study coordinating center. The patient is not obliged to give a reason for stopping participation.

2.4. Patient selection

60 patients with NSCLC stage IB-IV.

Inclusion criteria

- Non-small cell lung cancer stage IB-IV amenable for radiotherapy with curative intent.
- Patients not included in PET-boost trial
- WHO performance status 0-2
- Willing and able to comply with the study prescriptions
- 18 years or older
- Ability to give and having given written informed consent before patient registration
- No recent (< 3 months) severe cardiac disease (NYHA class >1) (congestive heart failure, infarction)
- No radiotherapy in 4 weeks prior to this study
- No treatment with investigational drugs in 4 weeks prior to or during this study.
- No known allergy to nitroglycerin or nitroglycerin patch.
- No known allergy to iodine based contrast agents
- No other active malignancy.
- No use of vaso-dilators (calcium channel blockers, nitrates or 5-fosfodiesterase inhibitors)
- No symptomatic hypotension
- No major surgery (excluding diagnostic procedures like e.g. mediastinoscopy) in previous 4 weeks.
- Adequate renal function: calculated creatinine clearance at least 60 ml/min.
2.5. Trial interventions: translational scanning

Step 1 and 2 are to be performed before the start of radiotherapy. Since preparation for radiotherapy usually takes 1.5 weeks between the intake and start, sufficient amount of time is left for these trial investigations.

2.5.1. Baseline imaging of tumor

Explanation: In this step we acquire baseline imaging of perfusion and oxygenation status of the tumors. After completion of the trial these results will be used to investigate whether it is possible to identify patients most likely to benefit from the addition of nitroglycerin to standard therapy.

The examined tumor will be the largest lesion (tumor or node) as assessed on CT scan.

Practical:

* Day 1:
  - Dynamic Contrast Enhanced (DCE) CT scan at radiology department before HX-4 scan
  - Injection of 444 MBq of HX-4 at nuclear medicine department
  - Static HX-4 PET-CT scan 240 minutes post-injection
2.5.2. Evaluating the effect of nitroglycerin

Explanation: By administering nitroglycerin prior to scanning and comparing this to the baseline scan, patients having most effect on the oxygenation status and perfusion of the tumour (and thus possibly likely to benefit from the addition of nitroglycerin) can be identified.

The baseline DCE-CT scans and the scans after nitroglycerin are to be kept a minimum of 2 days apart to avoid renal impairment by consecutive IV contrast infusions.

Practical:

Day 3/4:
- Nitroglycerin patch Transiderm Nitro 5 at 8.30 h.
- Dynamic Contrast Enhanced (DCE) CT scan at radiology department at 11.30 h.
- Injection of 444MBq of HX-4 at nuclear medicine department at 14 h.
- Static HX-4 PET-CT scan 240 minutes post-injection. Scan-time 20 minutes
- Removal of nitroglycerin patch after scanning is completed.

2.5.3. Evaluating response after treatment

Explanation: metabolic response on an 18 FDG PET-scan 70 days after therapy has been shown to have prognostic value in NSCLC. (27)

Practical: 18-FDG PET-CT scans will be planned 70 days +/- 10 days after the end of radiotherapy. This scan is not mandatory. Patients will be asked separately for permission to perform this scan. The PET scan should be made and calibrated according to the NEDPAS protocol, with an injected dose of FDG of 2.5 x Body Weight MBq. (28)
Scans will be interpreted according to the EORTC criteria for response assessment on FDG PET-scans (see table).
2.6. Trial interventions: Nitroglycerin + radiotherapy

Explanation: All patients will receive a Transiderm Nitro 5 patch daily during radiotherapy. The results of the scans before treatment will not have an influence hereon because the absence or presence of a predictive value of the baseline scans and the response of perfusion and oxygenation on nitroglycerin administration will only become clear after sufficient follow-up of the trial patients. Not only might nitroglycerin exert it’s effects through an effect on hypoxia, but other effects, such as stimulation of the immune response to the tumor, decrease of stimulating biomarkers (TGF beta, Hif 1 alpha) or stabilization of p53 has also been demonstrated in preclinical research. This will not be apparent on the scans, but might have an effect on the outcome of the trial nevertheless.

Patches are applied during the day because maximal concentrations of NO need to be present at the time of radiotherapy.

Nitroglycerin

Each patient will receive a nitroglycerin patch (Transiderm Nitro 5) containing 25 mg/10 cm² which releases nitroglycerin at a rate of 0.2 mg/h (which is the dose given by Yasuda et al and is a standard dose for treatment of angina pectoris).

The patch will be applied for 12 hours every day, from 7h-19h, this way avoiding the development of cardiovascular tolerance and ensuring maximal blood concentration of nitroglycerin and hence perfusion effect at each fraction of radiotherapy.
The patch cannot be applied at night because of the short half-life of approximately 1 hour of nitroglycerin in the body: when applied at night, no nitric oxide is present at the time of radiotherapy.

**Start of application**: on d1 (first day) of radiotherapy.

**Duration of application**: daily until last day of radiotherapy.

**Radiotherapy**:

All curative intent radiotherapy schedules in Maastro Clinic for stage Ib-IV NSCLC are allowed.

**Chemotherapy**:

All standard schedules for concurrent as well as neo-adjuvant chemotherapy containing cisplatin, etoposide, vinorelbine or taxanes are allowed.
2.6.1. Examinations:

**Before enrollment: Baseline examinations**
- Medical history
- Concomitant medication (Calcium antagonists? Beta blockers? Nitrates? Viagra/Levitra/Cialis?)
- Clinical examination (blood pressure, heart rate, WHO performance status, weight).
- Diagnostic FDG PET-CT (= standard for NSCLC to be treated with curative intent)
- Blood sample for renal function measurement (in the context of DCE-CT scans)

**At the day of patch application:**
- Clinical examination (blood pressure, heart rate) before patch.
- Clinical examination (blood pressure, heart rate) at patch removal.

**During radiotherapy: Weekly follow-up**  
**By Radiotherapist**
- CTCAE 4.0
- Clinical examination (WHO performance status, weight, blood pressure)

Remark: be sure to measure blood pressure in any case of complaints compatible with hypotension (dizziness, fatigue, fainting, orthostatism, ...)

**After radiotherapy: Monthly follow-up**  
**By Radiotherapist until resolution of complaints**
- CTCAE 4.0
- Clinical examination (blood pressure, heart rate, WHO performance status, weight)

**Follow-up after treatment: 3-monthly**  
**By referring physician**
- CTCAE 4.0
- Clinical examination (blood pressure, heart rate, WHO performance status, weight)
- Chest X-ray
- Scans when clinically indicated
Follow-up after treatment: 2.5 months (70 ± 10 days) after the end of radiotherapy
- 18-FDG PET-CT scan (voluntary)
2.6.2. Statistics:

Single-centre single arm, non-randomized combination phase II trial.

Number of included patients is calculated on the basis of the primary endpoint (2-year survival).

In a mixed population with 60 % stage III, 30 % stage I and 10 % stage II NSCLC treated at Maastro with individualized radiotherapy with or without sequential chemotherapy, 2-year survival after the date of diagnosis is roughly 46%. Stage I, II and III respectively reach an OS of 57 %, 32 % and 43 %.(29) The group of stage III patients treated with concurrent chemo-radiotherapy has a 2-year survival of 51.5 %. [unpublished data, Van Baardwijk et al, sent to JCO, see abstract in appendix]. Currently 2/3 of patients with stage III NSCLC receive concurrent chemo-radiation.

A minority of patients (<5% of all NSCLC cases) treated at Maastro Clinic receive radical radiotherapy for oligometastastic stage IV disease. Their overall survival at 2 years is estimated at 25 %. The overall 2-year survival of our currently treated patient population is thus estimated at 50 %.

The population described above by Van Baardwijk et al is representative of the normal patient population treated at Maastro Clinic. Every patient treated in Maastro Clinic for NSCLC stage I-III is irradiated using our standard regimen of accelerated, isotoxic radiotherapy. Each patient is prospectively enrolled in a database through the electronical medical file, which is linked to the National Registry, ensuring complete survival data for all patients treated at Maastro Clinic. The patient population eligible for this trial roughly consists of all patients referred to Maastro Clinic for these standard radiotherapy regimens, excluding patients with poor renal function or recent severe cardiac problems, which is considered to be a minority of patients since a large proportion of these patients is not deemed apt to undergo radical therapy beforehand and will thus never enter Maastro Clinic. Thus, we believe the normal patient population of Maastro provides a robust denominator for statistical comparison.
In this phase II trial we aim at demonstrating an absolute rise in 2-year survival of 15% of the addition of nitroglycerin to standard radiotherapy, thus increasing 2-year survival for the whole group from 50% to 65%.

Statistical evaluation of the primary endpoint is based on an exact, single sided confidence interval. Thus, the number of patients alive at 2-years after diagnosis will be counted, and the one-sided confidence interval with alpha of 0.1 will be calculated. Given the survival percentage of 50% for standard treatment, the confidence interval should exclude this value. The sample size for the study can be calculated by extension of this principle. Using an alfa-value of .10 and a power of .80, a standard survival percentage of 50% and an expected improvement to 65%, we would thus need a minimum of 53 patients of which 32 should be alive at 2 years after diagnosis. To compensate for patients potentially dropping out before treatment start, we will aim at 60 patients to be included in the trial.(30)

Accrual of patients is conservatively expected to run over 4 years, including 15 patients a year. At Maastro Clinic several trials have run in the same patient population in past years. Accrual in the aforementioned trial of Van Baardwijk et al (29) was 166 patients with stage I-III NSCLC from December 2004 to June 2007. The follow-up trial in stage III patients accrued 150 stage III patients fit for concurrent chemo-radiation from April 2006 till December 2009.

The accrual for this trial is expected to be slower because of 2 reasons: the currently running PET-boost trial in stage I-III NSCLC and the fact patients are expected to undergo 4 extra scans on 2 separate days before treatment start, which is an extra burden possibly hampering accrual.
2.6.3. Toxicity follow-up and early stopping rule

**Safety Monitoring**
Adverse events will be monitored on an ongoing basis and their frequencies reported annually to the Medical Ethical Committee of the Academic Hospital Maastricht. Toxic effects will be categorized using the NCI Common Terminology Criteria for Adverse Events, Version 4.0. The worst event for each patient will be described. Both events related and unrelated to treatment will be captured.

Clinical and laboratory data will be tabulated and compared to normal ranges for the institution.

During radiotherapy patients are clinically checked on a weekly basis.

Acute toxicity follow-up by the radiotherapist will take place at 4 weeks post-radiotherapy and is continued by the radiotherapist on a monthly basis until resolution of acute toxicity.

The follow-up will be continued afterwards by the pulmonologist in the respective referring institution.

Patients will receive the standard follow-up questionnaires used in Maastro Clinic for follow-up of late toxicity.

**Toxicity to be systematically recorded/graded:**
Radiotherapy:
- Dyspnea
- Dysphagia
- Cough
**Early stopping rule**

In the Van Baardwijk series (29) of radical radiotherapy of stage I-III NSCLC the occurrence of grade 3 or more acute dyspnea, cough and dysphagia was respectively 10%, 12% and 5%.

For concurrent chemoradiation in stage III NSCLC, these respective incidences are 3.5%, 8.5% and 24% [unpublished data].

Overall an incidence of 20% grade 3 or more dyspnea, 20% or more grade 3 cough and 30% or more grade III dysphagia is deemed unacceptable.

To prevent us from taking forward a regimen which is too toxic, after 30 patients an interim analysis will be performed.

In case 6 patients or more have experienced grade 3 or more dyspnea or cough and 10 or more patients have encountered grade 3 or more dysphagia, recruitment of patients will be stopped at this point.
3  **Study administration and management**

The data-centre of MAASTRO CLINIC will do the study administration and management. They will also have access to the source data, the subject files and do the archiving of all items that are necessary for reviewing the data of the study and ensuring Quality Control.

4  **Study report**

When the primary endpoint has been reached, a study report will be written. The aim is to report the study findings in an international peer-reviewed journal and to present them at international meetings.
Also interim results can be published on toxicity, tumour hypoxia and tumour perfusion.
5 Responsibilities investigators:

The investigators undertake to conduct the clinical trial according to the protocol approved by the Ethics Committee. The investigators may not change any of the terms of the protocol without the agreement of the Ethics Committee. All the investigators should be GCP certified.

The principal investigator is responsible for:
- Identifying those members of his/her team who are to participate in the study and defining their responsibilities;
- Ensuring, as far as possible, that the required numbers of patients are enrolled in the trial, within the time period established for such inclusion.

Each investigator is responsible for:
- Collecting informed consent forms, duly signed and dated by the patients, prior to any specific trial selection procedures;
- Ensuring that the Clinical Research Assistant (CRA) has direct access to all the original documents, so that the latter may validate observation sheet data;
- Agreeing to regular visits from the CRA and where applicable, those of auditors appointed inspectors from the supervisory authorities.

All documents relating to the study (protocol, consent forms, CRFs, the investigator file and so on), together with original documents (laboratory results, x-rays, consultation reports, clinical examination reports, etc.) are to be kept in a safe place and are to be considered as confidential.

Archiving of data will be the investigator’s responsibility and is to be carried out according to legislation currently in force. The investigator must keep the study data together with a list of patients for at least 15 years after the end of the study.

The duties mentioned above for the investigators may be delegated to the datamanagers or research nurses involved in the study.
In accordance with the Directive 2001/20/EC requirements, it is the principle investigator`s (Philippe Lambin) responsibility:
- To take out civil liability insurance against consequences of the research that may be prejudicial for the person taking part in it;
- To submit all the documents required to the Ethics Committee (EC) and the Competent Authority (CA) according to local requirements;
- To obtain the approval from the Ethics Committee and the Competent Authority before starting the trial
- To inform the managers of the health institutions;
- To transmit all information relevant to the management of the research to the investigators
- To provide sufficient material to permit the investigators to conduct the trial according to the agreed protocol.

The P.I. must ensure that documents vital to the running of the trial are safely archived for the minimum 15-year period provided for by Good Clinical Practice guidelines, that is to say, 15 years after the end of the research activity.
6 Ethical considerations

6.1. Risk

No delay of regular anti-cancer therapy is required for this study.

6.2. Ethical Committee

Approval of the medical ethical committee (MEC) will be obtained before start of the trial.

6.3. Subject identification

A random code will be attributed to each patient registered in the trial. This number will identify the patient and must be included on all case report forms. The number will consist of 3 randomly chosen letters which will be attributed by the datamanagement of MAASTRO CLINIC and linked to the patient in a list kept by datamanagement only. This list can be viewed by the responsible physician and if necessary, the (national) regulatory institution. The minimum duration of the storage of the study-data is 15 years.

6.4. Informed consent

Patients will be informed about this trial by the radiation oncologist or pulmonologist. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. The patient informed consent statement is given as an appendix to this protocol. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient’s subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered or randomized in the study. This must be
done in accordance with the national and local regulatory requirements. The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that “the written informed consent form should be signed and personally dated by the patient”. Eligible patients will receive information about this study from the physician on intake (mostly the radiotherapist) and will at that time also receive a written patient information and informed consent forms. Patients have at least three days time to consider participation in the study. When the patient decides to participate in the study, he or she will date and sign the informed consent form, sometimes after orally consenting to participation. The physician/researcher also dates and signs this informed consent. These signing dates are not necessarily on the same day, due to the fact that patients should not have to come to MAASTRO clinic only to hand in their informed consent form. Moreover, planning of examinations and treatment can start when oral consent is given. Before any study-related actions take place, the written informed consent must be given to the physician. An independent physician is appointed for this study: Dr. Roy Lalisang. He can answer all questions about the study patients would rather not ask their physician.
6.5. Assessment and reporting of safety

6.5.1. Section 10 WMO event
In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects’ health. The investigator will take care that all subjects are kept informed.

6.5.2. Adverse and serious adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to nitroglycerin treatment. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that at any dose: - results in death
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients’ hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs will be reported through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days
Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product).

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:
- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.
6.5.4. Annual safety report
In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, Medicine Evaluation Board and competent authorities of the concerned Member States. This safety report consists of:
- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

6.5.5. Follow-up of adverse events
All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
7 Insurance

Participants are insured against any harm due to the investigation, both by a Clinical Trials Insurance and a Public Liability Insurance, according to WMO.

8 Publication policy

When the primary endpoint has been attained a study report will be written. The aim is to report the study findings in international peer-reviewed journals and to present it at international meetings. Interim analyses can be published after each cohort of 15 patients. Results of DCE-CT-scanning and HX-4 scanning can be reported separately.

The junior clinical investigator (Bart Reymen) will be first author on all papers. The coordinator (Dirk De Ruyscher) will be second author and last author will be the principle investigator (Philippe Lambin). Marco Das and Joachim Wildberger will be listed as co-authors on all papers concerning DCE-CT scanning and Eric Vegt will be listed as co-author on all papers concerning HX-4 scanning.

9 Potential benefits or detriments

Patients are not expected to have any benefit from this trial, since no change in regular anti-cancer therapy is performed.

Potential risks: the extra burden in this trial consists of 2 DCE CT scans and 2 HX-4 PET-scans.

Each DCE-CT scan will add on average 7-7.5 mSV to the radiation dose received (ng et al). Each HX-4 PET-CT scan will add on average 20-25 mSV (see above) depending on voiding frequency.

When compared to the 65000 mGy which is on average administered to patients treated with radiotherapy for NSCLC at Maastro Clinic, this added radiation dose by the extra scans is negligible.
Contrast enhancement has the known risks of allergy and renal failure, which is generally a low risk. To minimize the risk of renal impairment by a bolus injection of contrast used in DCE-CT scanning, the perfusion CT scans are performed with a minimal interval of 48 hours in between.

Nitroglycerin has as most common side effects: headache (60% of patients), light-headedness (6% of patients), syncope (4% of patients).

Theoretically, the addition of nitroglycerin to radiotherapy might alter tissue perfusion, increasing the radiosensitivity of irradiated normal structures and enhancing side effects of radiotherapy. However, since nitrates are widely used in cardiovascular medicine and no reports can be found on Pubmed on increased radiation side effects by nitroglycerin, this risk is perceived to be low by the investigators.

10 Conflicting trials

As mentioned in the inclusion criteria, patients included in the PET-boost trial cannot be included in the present trial.

The PET-boost (or BTV-boost) trial is a trial conducted at Maastro Clinic and the NKI investigating the feasibility and effect on survival of redistributing and escalating the radiotherapy dose to resistant areas inside the primary tumor based on FDG-PET uptake. The main trial inclusion criteria are:

- stage I-III pathologically proven NSCLC
- with an SUV of the primary tumor $\geq 5$
- with a minimal tumor diameter of 4 cm
- sent for radical radiotherapy $\pm$ chemotherapy

Since in the PET-boost trial radiotherapy doses are delivered which far exceed the standard radiotherapy doses delivered in everyday practice, there might be an interference with the survival and toxicity evaluation. Also, systematic addition of nitroglycerin and the possible effect thereof on the tumor might interfere with the results of the BTV boost trial.
Therefore inclusion in both trials is prohibited.

Since however the PET-boost trial is a technically very sophisticated and logistically highly demanding trial (the preparation/ calculation of 1 plan takes on average 1.5 weeks vs 2 days in normal practice for a NSCLC patient) the inclusion of patients is for logistical reasons restricted to 2 simultaneous patients at any given time in the preparation process at Maastro. This comes down to roughly 2 patients per month. The rest of all NSCLC patients treated at Maastro can theoretically be considered for inclusion in the nitroglycerin trial. Thus, the effect of the PET-boost trial on the inclusion rate of the nitroglycerin trial is believed to be limited.

Recently we have been informed of another trial currently running in the AZM in patients with NSCLC: the Lucanix trial. This is an international, randomized multicenter trial investigating the effect of Lucanix (belagenpumatucel-L, an anti-tumor vaccine) in patients with at least stable disease after platinum based chemotherapy for (stage cT3N2 up to stage 4) NSCLC.

Main inclusion Criteria:
Subjects with histologically or cytologically confirmed NSCLC who meet one of the following staging requirements:
Stage IIIA (T3N2 only) or Stage IIIB or Stage IV.
Subjects must have stable disease (SD) or an objective response (PR or CR) to a prior single, frontline, platinum-based chemotherapy regimen (additional prior adjuvant chemotherapy is permitted) consisting of up to six (6) treatment cycles with or without concomitant radiation therapy.
Not less than four weeks nor more than four months must have elapsed since the completion of the last chemotherapy cycle and registration into the study.
Subjects treated for brain metastasis(es) are eligible if they have been stable for ≥ 2 months.
Performance status (ECOG) ≤ 2.
Adequate hematological, renal and liver tests
Since this trial is mainly recruiting patients in palliative setting and the AZM is the only regional hospital enrolling, the impact of this trial on our inclusion rate is perceived to be minimal. Furthermore: patients can only be enrolled after documented stable disease at the end of chemotherapy, meaning inclusion in the nitroglycerin trial has already occurred. On top of that, the target for inclusion of patients of the AZM has already been achieved, meaning this sponsored trial is currently still running to include extra patients for the overall trial population, but will be closed in a couple of months. The overlap with the nitroglycerin trial will thus be minimal. Patients can not be included in both trials since nitroglycerin could in theory influence the immune-effects related to the vaccine and vice-versa.

11 Notification to authorities
The study will be notified to the CCMO, according to Dutch laws and regulations.

12 Trial registries
The study will be registered in the CCMO registry in the Netherlands as well as in the Cancer Trial Registry of the National Cancer Institute of the USA.

13 Law
This trial complies with the Declaration of Helsinki and the WMO (Wet Medisch-Wetenschappelijk Onderzoek).
14 Appendices

14.1. WHO performance status scale

0 Able to carry out all normal activity without restriction
1 Restricted in physically strenuous activity but ambulatory and able to carry out light work.
2 Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.
3 Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4 Completely disabled; cannot carry out any self-care; totally confined to bed or chair.

14.2. Nitroglycerin patch: Instructions for use

- Nitroglycerin Patch is for external use only.
- Wash hands thoroughly before and after applying the patch.
- Apply the patch to a non-hairy area of the chest, inner side of the upper arm, back or shoulder.
- Clean and completely dry the skin before applying the patch.
- If necessary, hair should be removed by clipping or lightly shaving.
- Remove the patch from the package.
- Apply with a firm pressure to the skin.
- Do not apply to irritated or damaged skin.
- After removing the used patch, fold it in half with the sticky sides together.
- Discard the patch out of the reach of children and away from pets.
15 Appendix: HX-4 PET, perfusion CT, Nitroglycerin

15.1. Appendix A. 18F-HX4 PET-CT

Production and Quality aspects of [18F]HX4 conjugates

Radiolabeling of HX-4 with $^{18}$F will be performed at the Radionuclide Center (RNC) of the Department of Nuclear Medicine & PET research of the VuMC Amsterdam. The whole process will be performed according to state-of-the-art Good Manufacturing Practice (GMP) standards. This production site is licensed to manufacture tracers for human use according to the latest EU guidelines. This site has also been audited by the largest EU pharma companies.

The manufacturing of the final quality-assured $[^{18}F]$HX4 IP occurs in 3 steps. The first step incorporates a nucleophilic substitution of radioactive fluorine (in the form of $^{18}$F) onto the precursor material, yielding the active drug substance. Secondly, the fluorinated intermediate is hydrolyzed to obtain the active drug product. Finally, the obtained product is purified by semiprep HPLC and formulated into an human applicable solution.

In general, the expiration time $[^{18}F]$HX4 is 4-5 hours. Like other short-lived positron emitting radiotracers for PET-CT, $[^{18}F]$HX-4 must be manufactured in carefully controlled batches at times close to the time of the scheduled PET imaging procedure. The product, ready for injection, will be delivered in a 20 ml sterile vial.

Each dose of $[^{18}F]$HX4 is packaged and labelled for a specific study subject, and delivered to the AZM nuclear medicine department prior to PET imaging. Each dosing package includes three items:
- Sterile vial with with a solution of $[^{18}F]$HX4 in approximately 5% ethanol in phosphate buffer containing approximately 50 mg/ml ascorbic acid
- Surrounding leaded vial carrier
- Outside shipping canister, certified for the transport of $[^{18}F]$labelled products

Every $[^{18}F]$HX4 batch will meet the specifications summarized in the table below:
Specifications $[^{18}\text{F}]\text{HX4}$ batches

| Pre-Release specifications                        |   |
|--------------------------------------------------|---|
| Appearance                                       | Clear solution free of particulate matter |
| pH                                               | 5 to 7.5 |
| Radiochemical Purity                             | $\geq 95\%$ |
| Radiochemical Identity                           | Rt corresponds with HX4 Reference standard |
| Radioactivity Concentration                      | $\geq 1\text{-}30\text{ mCi/mL @ EOS}$ |
| Radionuclidic Identity                           | $T_{1/2} = 105\text{-}115\text{ minutes}$ |
| $[^{18}\text{F}]\text{HX4}$ Specific Activity   | $\geq 400\text{ mCi/µmol}$ |
| K222 Limit Test                                  | $\leq 50\text{ µg/mL}$ |
| Bacterial Endotoxin                              | $\leq 175\text{ EU/dose}^*$ |
| Residual Solvents                                | Acetonitrile: $\leq 0.04\%\text{ w/w}$ |
|                                                  | Ethanol: 1.0\text{-}6.0\% w/w |
| Membrane Filter Integrity                        | Millex: $\geq 50\text{ psig}$; |
|                                                  | Pall: $\geq 46\text{ psig}$ |

| Post-Release Test specifications                 |   |
| Sterility                                        | Meets current USP <71> Sterility Test Require |
| Radionuclidic Purity (Performed monthly on decayed sample) | $\geq 99.9\%\text{ F-18}$ |

**Dosage and Treatment Schedule**

**Packaging, labeling and supply**

HX4 will be appropriately labeled with $^{18}\text{F}$ for investigational use and will be delivered directly from VU Medical Center to the department of Nuclear Medicine of the azM.

IMP receipt, drug accountability reporting forms and reconciliation or disposal procedures should be maintained separately from this protocol, and will be reviewed with the Investigator during periodic monitoring visits.
Storage conditions
The unlabelled HX-4 will be stored in the hospital pharmacy of the VUMC in a limited access area for materials at a monitored temperature between +2 and +8 degrees Celsius. With regard to the labelled [18F]HX4, 4-5 hours may elapse between radiolabeling of the compound and administration to the patient.

HX-4 infusion
Qualified site personnel will administer [18F]HX4 to the patient, assay and record the dose of [18F]HX4 prior to injection. Assay and record residual activity after injection. [18F]HX4 is administered to the patient via an intravenous catheter line, followed by an adequate amount of saline flush (about 10 cc). Individual doses of [18F]HX4 will contain a maximum of 444MBq (12 mCi).

Patient instructions
The urinary bladder wall receives the highest dose of 18F-HX4 and is the critical organ.(31) Therefore patients are encouraged to maintain adequate hydration and void frequently. Before the scan the patient has to confirm that he/she has voided.

Imaging procedure
HX-4 PET-CT studies will be performed at the Maastricht University Medical Center using the Philips Gemini TF PET/CT with a 64 slice Brilliance CT component (Philips, Medical Systems, Best, The Netherlands). The scanner has a transaxial field-of-view (FOV) of 57.6 cm and an axial FOV of 18 cm. Its PET scanner consists of 44 rings of Lutetium-Yttrium Oxyorthosilicate (LYSO) crystal detectors with time-of-light capability (650 ps time resolution (FWHM)). Its spatial resolution is approximately 5 mm (FWHM). Data acquisition is in 3D yielding a high sensitivity but requiring a good scatter correction. During reconstruction CT based attenuation and scatter corrections are applied, including correction for dead time and decay. PET-CT images are reconstructed using Ordered Subset Expectation Maximization (OSEM). Slice thickness and spacing for PET are 2.0 mm; the voxel size is 2x2x2 mm³. The scanner is able to perform list-mode acquisition for dynamic or respiratory-correlated PET and CT imaging.
During the acquisition, no contrast will be used.
Patients are positioned in supine position, with their arms in an arm support. The head of the patient is in retro-flexion on a head base. A knee pillow is used. When clinically indicated, individualized adjustment of the positioning as well as the immobilization devices are allowed.
**Data storage**

All planar images and tomographic data will be stored permanently in MAASTRO’s data archive system, and a coded copy (random number), in which the patient identity cannot be determined, will be provided to the Sponsor for permanent record.

**Image analysis and evaluation**

The analysis and evaluation of the images will be done using the state-of-the-art multi-modality workstation Siemens TrueD. Tumor ROI’s are drawn on the registered PET-CT images and a background region in unirradiated muscle tissue is drawn (preferably in the paraspinal muscular area of the upper thoracic spine) to determine a tumor to background ratio. A threshold is applied to the total tumor ROI to determine a hypoxic fraction and to spatially compare high uptake FDG regions with hypoxic areas.

All regions should be saved on the computer, in order to project the ROI’s on images at other time points. The pixel size and the number of pixels in each region should be reported. The number of counts in the regions for all imaging time points should be recorded digitally in a spreadsheet.

Special attention will be given to the kinetic behaviour in each tissue (especially the tumor) by comparing the activity concentrations at the different time points. Kinetic modelling will be performed with present software (Philips Imalytics Workspace or PMOD).
15.2. Appendix B. Imaging protocol CT perfusion

Perfusion CT scans will be performed at the Radiology department of the University Medical Centre Maastricht. A Siemens Somatom Definition Flash CT will be used to acquire perfusion CT images.

VOLUMETRIC PERFUSION CT SEQUENCE:

4D Spiral mode:
80kV, 120 mAs (care dose OFF)
13.7cm coverage:
Scan delay from IV contrast – 5 seconds
Cycle time 1.75 seconds between acquisitions for first 30 seconds
Number of scans - 17
Breathhold interval
Then Cycle time 3.5 seconds between acquisitions for next 30 seconds
Number of scans – 9
Then cycle time 5.25 seconds between acquisitions for next 30 seconds
Total acquisition time – 91.93 seconds
Estimated CTDIvol – 71.43mGy
Detector collimation: 24 X1.2mm
Rotation speed: 0.33 seconds
Scan time 1.8seconds
Reconstruction: 1.5, 3 and 5mm slice thickness with an increment of 1, 2 and 3mm respectively, reconstruction kernel B20f smooth

Contrast-media:

70 mL Ultravist 300 mg/mL iodine; Bayer-Schering, Berlin, Germany at 7mL/s followed by saline chaser 50mL/7 ml/s
Parameters to be determined:

- Tumor Blood Volume
- Tumor Permeability
15.3. Nitroglycerin FDA label

15.3.1. Nitroglycerin Transdermal Description

Nitroglycerin is 1,2,3-propanetriol trinitrate, an organic nitrate whose structural formula is:

\[
\begin{align*}
H_2C\text{ONO}_2 \\
H\text{CNO}_2 \\
H_2\text{CNO}_2
\end{align*}
\]

and whose molecular weight is 227.09. The organic nitrates are vasodilators, active on both arteries and veins.

The Nitroglycerin Transdermal System is a flat unit designed to provide continuous controlled release of nitroglycerin through intact skin. The rate of release of nitroglycerin is linearly dependent upon the area of the applied system; each cm² of applied system delivers approximately 0.03 mg of nitroglycerin per hour. Thus, the 7-, 14-, and 21-cm² systems deliver approximately 0.2, 0.4 and 0.6 mg of nitroglycerin per hour, respectively.

The remainder of the nitroglycerin in each system serves as a reservoir and is not delivered in normal use. After 12 hours, for example, each system has delivered approximately 6% of its original content of nitroglycerin.

15.3.2. Clinical Pharmacology

The principal pharmacological action of nitroglycerin is relaxation of vascular smooth muscle and consequent dilatation of peripheral arteries and veins, especially the latter. Dilatation of the veins promotes peripheral pooling of blood and decreases venous return to the heart, thereby reducing left ventricular end-diastolic pressure and pulmonary capillary wedge pressure (preload). Arteriolar relaxation reduces systemic vascular resistance, systolic
arterial pressure, and mean arterial pressure (afterload). Dilatation of the coronary arteries also occurs.

The relative importance of preload reduction, afterload reduction, and coronary dilatation remains undefined.

Dosing regimens for most chronically used drugs are designed to provide plasma concentrations that are continuously greater than a minimally effective concentration. This strategy is inappropriate for organic nitrates.

Several well-controlled clinical trials have used exercise testing to assess the anti-anginal efficacy of continuously-delivered nitrates. In the large majority of these trials, active agents were indistinguishable from placebo after 24 hours (or less) of continuous therapy. Attempts to overcome nitrate tolerance by dose escalation, even to doses far in excess of those used acutely, have consistently failed. Only after nitrates have been absent from the body for several hours has their anti-anginal efficacy been restored.
15.3.3. Pharmacokinetics

The volume of distribution of nitroglycerin is about 3 L/kg, and nitroglycerin is cleared from this volume at extremely rapid rates, with a resulting serum half-life of about 3 minutes. The observed clearance rates (close to 1 L/kg/min) greatly exceed hepatic blood flow; known sites of extrahepatic metabolism include red blood cells and vascular walls. The first products in the metabolism of nitroglycerin are inorganic nitrate and the 1,2- and 1,3-dinitroglycerols. The dinitrates are less effective vasodilators than nitroglycerin, but they are longer-lived in the serum, and their net contribution to the overall effect of chronic nitroglycerin regimens is not known. The dinitrates are further metabolized to (non-vasoactive) mononitrates and, ultimately, to glycerol and carbon dioxide.

To avoid development of tolerance to nitroglycerin, drug-free intervals of 10-12 hours are known to be sufficient; shorter intervals have not been well studied. In one well-controlled clinical trial, subjects receiving nitroglycerin appeared to exhibit a rebound or withdrawal effect, so that their exercise tolerance at the end of the daily drug-free interval was less than that exhibited by the parallel group receiving placebo.

In healthy volunteers, steady-state plasma concentrations of nitroglycerin are reached by about two hours after application of a patch and are maintained for the duration of wearing the system (observations have been limited to 24 hours). Upon removal of the patch, the plasma concentration declines with a half-life of about an hour.

This is the reason why nitroglycerin in the current trial cannot be delivered during the night as is customary in patients with cardiovascular disease: upon radiotherapy, patients would not have sufficient blood concentrations of nitroglycerin to exert an effect on the tumour vasculature.

15.3.4. Contraindications

Use of Nitroglycerin Transdermal system is contraindicated in patients using Sildenafil (Viagra) because sildenafil may amplify the vasodilatory effects of Nitroglycerin Transdermal systems resulting in severe hypotension.

Similar warnings apply to Vardenafil (Levitra) and Tadalafil (Cialis).
Allergic reactions to organic nitrates are extremely rare, but they do occur. Nitroglycerin is contraindicated in patients who are allergic to it. Allergy to the adhesives used in nitroglycerin patches has also been reported, and it similarly constitutes a contraindication to the use of this product.

15.3.5. Warnings

Amplification of the vasodilatory effects of nitroglycerin by Sildenafil (Viagra) can result in severe hypotension. The time course and dose dependence of this interaction have not been studied. Appropriate supportive care has not been studied, but it seems reasonable to treat this as a nitrate overdose, with elevation of the extremities and with central volume expansion.

Similar warnings apply to Vardenafil (Levitra) and Tadalafil (Cialis).

A cardioverter/defibrillator should not be discharged through a paddle electrode that overlies a Nitroglycerin Transdermal patch. The arcing that may be seen in this situation is harmless in itself, but it may be associated with local current concentration that can cause damage to the paddles and burns to the patient.
15.3.6. Precautions

General

Severe hypotension, particularly with upright posture, may occur with even small doses of nitroglycerin. This drug should therefore be used with caution in patients who may be volume depleted or who, for whatever reason, are already hypotensive. Hypotension induced by nitroglycerin may be accompanied by paradoxical bradycardia and increased angina pectoris.

Nitrate therapy may aggravate the angina caused by hypertrophic cardiomyopathy. As tolerance to other forms of nitroglycerin develops, the effect of sublingual nitroglycerin on exercise tolerance, although still observable, is somewhat blunted.

In industrial workers who have had long-term exposure to unknown (presumably high) doses of organic nitrates, tolerance clearly occurs. Chest pain, acute myocardial infarction and even sudden death have occurred during temporary withdrawal of nitrates from these workers, demonstrating the existence of true physical dependence.

Several clinical trials in patients with angina pectoris have evaluated nitroglycerin regimens which incorporated a 10-12 hour nitrate-free interval. In some of these trials, an increase in the frequency of anginal attacks during the nitrate-free interval was observed in a small number of patients. In one trial, patients had decreased exercise tolerance at the end of the nitrate-free interval. Hemodynamic rebound has been observed only rarely; on the other hand, few studies were so designed that rebound, if it had occurred, would have been detected. The importance of these observations to the routine, clinical use of transdermal nitroglycerin is unknown.

15.3.7. Information for Patients

Daily headaches sometimes accompany treatment with nitroglycerin. In patients who get these headaches, the headaches may be a marker of the activity of the drug. Patients should resist the temptation to avoid headaches by altering the schedule of their treatment
with nitroglycerin, since loss of headache may be associated with simultaneous loss of efficacy.

Treatment with nitroglycerin may be associated with lightheadedness on standing, especially just after rising from a recumbent or seated position. This effect may be more frequent in patients who have also consumed alcohol.

After normal use, there is enough residual nitroglycerin in discarded patches that they are a potential hazard to children and pets.

15.3.8. Drug Interactions

The vasodilating effects of nitroglycerin may be additive with those of other vasodilators. Alcohol, in particular, has been found to exhibit additive effects of this variety.

Marked symptomatic orthostatic hypotension has been reported when calcium channel blockers and organic nitrates were used in combination. Dose adjustments of either class of agents may be necessary.

15.3.9. Adverse Reactions

Adverse reactions to nitroglycerin are generally dose-related, and almost all of these reactions are the result of nitroglycerin's activity as a vasodilator.

Headache, which may be severe, is the most commonly reported side effect. Headache may be recurrent with each daily dose, especially at higher doses.

Transient episodes of lightheadedness, occasionally related to blood pressure changes, may also occur.

Hypotension occurs infrequently, but in some patients it may be severe enough to warrant discontinuation of therapy.
Syncope, crescendo angina, and rebound hypertension have been reported but are uncommon.

Allergic reactions to nitroglycerin are also uncommon, and the great majority of those reported have been cases of contact dermatitis or fixed drug eruptions in patients receiving nitroglycerin in ointments or patches.

There have been a few reports of genuine anaphylactoid reactions, and these reactions can probably occur in patients receiving nitroglycerin by any route.

Extremely rarely, ordinary doses of organic nitrates have caused methemoglobinemia in normal-seeming patients. Methemoglobinemia is so infrequent at these doses that further discussion of its diagnosis and treatment is deferred.

Application-site irritation may occur but is rarely severe.
In two placebo-controlled trials of intermittent therapy with nitroglycerin patches at 0.2 to 0.8 mg/hr, the most frequent adverse reactions among 307 subjects were as follows:

|                          | placebo | patch |
|--------------------------|---------|-------|
| headache                 | 18%     | 63%   |
| lightheadedness          | 4%      | 6%    |
| hypotension and/or syncope| 0%      | 4%    |
| increased angina         | 2%      | 2%    |

### 15.3.10. Overdosage

**Hemodynamic Effects**

The ill effects of nitroglycerin overdose are generally the results of nitroglycerin's capacity to induce vasodilatation, venous pooling, reduced cardiac output, and hypotension. These hemodynamic changes may have protean manifestations, including increased intracranial pressure, with any or all of persistent throbbing headache, confusion, and moderate fever, vertigo; palpitations; visual disturbances; nausea and vomiting (possibly with colic and even bloody diarrhea); syncope (especially in upright posture); air hunger and dyspnea, later followed by reduced ventilatory effort; diaphoresis, with the skin either flushed or cold and clammy; heart block and bradycardia; paralysis; coma; seizures; and death.

Laboratory determinations of serum levels of nitroglycerin and its metabolites are not widely available, and such determinations have, in any event, no established role in the management of nitroglycerin overdose.

No data are available to suggest physiological maneuvers (e.g., maneuvers to change the pH of the urine) that might accelerate elimination of nitroglycerin and its active metabolites. Similarly, it is not known which - if any - of these substances can usefully be removed from the body by hemodialysis.

No specific antagonist to the vasodilator effects of nitroglycerin is known, and no intervention has been subject to controlled study as a therapy of nitroglycerin overdose. Because the hypotension associated with nitroglycerin overdose is the result of
venodilatation and arterial hypovolemia, prudent therapy in this situation should be directed toward increase in central fluid volume.

Passive elevation of the patient's legs may be sufficient, but intravenous infusion of normal saline or similar fluid may also be necessary.

The use of epinephrine or other arterial vasoconstrictors in this setting is likely to do more harm than good.

In patients with renal disease or congestive heart failure, therapy resulting in central volume expansion is not without hazard. Treatment of nitroglycerin overdose in these patients may be subtle and difficult and invasive monitoring may be required.

Methemoglobinemia

Nitrate ions liberated during metabolism of nitroglycerin can oxidize hemoglobin into methemoglobin. Even in patients totally without cytochrome b5 reductase activity, however, and even assuming that the nitrate moieties of nitroglycerin are quantitatively applied to oxidation of hemoglobin, about 1 mg/kg of nitroglycerin should be required before any of these patients manifests clinically significant (≥10%) methemoglobinemia.

In patients with normal reductase function, significant production of methemoglobin should require even larger doses of nitroglycerin. In one study in which 36 patients received 2-4 weeks of continuous nitroglycerin therapy at 3.1 to 4.4 mg/hr, the average methemoglobin level measured was 0.2%; this was comparable to that observed in parallel patients who received placebo.

Notwithstanding these observations, there are case reports of significant methemoglobinemia in association with moderate overdoses of organic nitrates. None of the affected patients had been thought to be unusually susceptible.

Methemoglobin levels are available from most clinical laboratories. The diagnosis should be suspected in patients who exhibit signs of impaired oxygen delivery despite adequate cardiac output and adequate arterial pO2. Classically, methemoglobinemic blood is described as chocolate brown, without color change on exposure to air.
When methemoglobinemia is diagnosed, the treatment of choice is methylene blue, 1-2 mg/kg intravenously.

This information was derived of the original FDA label of nitroglycerin, April 2000.
List of abbreviations

CA: Competent Authority
cGMP: cyclic Guanyl Mono Phospate
CRA: Clinical Research Assistant
CRF: complication report form
CT: Computed Tomography
CTCAE: Common Terminology Criteria for Adverse Events
DU: Doppler Ultrasound
DCE-CT: Dynamic Contrast Enhanced Computed Tomography or perfusion CT
ECG: electrocardiogram
EPR: Electron paramagnetic resonance
EC: Ethics Committee
$^{18}$F: 18-Fluorine labeled
$^{18}$F MISO: $[^{18}$F$]$Fluoromisonidazole
$^{18}$F HX-4: $3-[^{18}$F$]$fluoro-$2-(4-((2$-nitro$-1H$-imidazol$-1$-yl)methyl)$-1H$-1,2,3$-triazol$-1$-yl)$propan$-1$-ol
$^{18}$F EF3: $^{18}$F 2-(2$-Nitroimidazol$-1$-yl)$-N$-(3,3,3-trifluoropropyl)$-acetamide
$^{18}$F FAZA: $^{18}$F labeled azomycin-arabinoside
Hif-1 alpha: hypoxia inducible factor alpha-1
MBq: Mega Becquerel
MRI: Magnetic Resonance Imaging
mtADH: mitochondrial aldehyde dehydrogenase
NO: Nitric Oxide
NSCLC: non-small cell lung cancer
NYHA: New York Heart Association
PET: Positron Emission Tomography
pi: post-injection
pO2: oxygen pressure
PSA: prostate specific antigen
TGF-beta: transforming growth factor beta
VEGF: vascular endothelial growth factor
WMO: Wet Medisch-Wetenschappelijk Onderzoek
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RESULTS OF A PHASE II TRIAL ON INDIVIDUALIZED RADIATION DOSE-ESCALATION BASED ON NORMAL TISSUE CONSTRAINTS IN CONCURRENT CHEMO-RADIATION FOR STAGE III NON-SMALL CELL LUNG CANCER (NSCLC) (NCT00572325 TRIAL)

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Abstract
Purpose: Sequential individualized isotoxic accelerated chemo-radiation has been shown to be effective in non-small cell lung cancer (NSCLC), allowing delivering of high biological doses. We therefore performed a phase II trial (NCT00572325) investigating the same strategy in concurrent chemo-radiation in stage III NSCLC.

Patients and methods: 150 stage III patients fit for concurrent chemo-radiation (PS 0-2; FEV1 and DLCO ≥30%) were included from April 2006 till December 2009. An individualized prescribed dose based on normal tissue dose constraints was applied: mean lung dose (MLD) 19 Gy, spinal cord 54 Gy, brachial plexus 66 Gy, central structures 74 Gy. A total dose between 51 and 69 Gy was delivered in 1.5 Gy BID up to 45 Gy, followed by 2 Gy QD. Radiotherapy was started at the 2nd or 3rd course of chemotherapy. Primary endpoint was overall survival (OS) and secondary endpoint toxicity (CTCAE v3.0).

Results: The median tumor volume was 73.9±91.3 cc; 50.0% of patients had N2 and 31.3% N3 disease. The median dose was 65.0±5.9 Gy delivered in 35±5.6 days. Six patients (4.0%) did not complete radiotherapy. With a median follow-up of 29.4 months, the median OS was 24.2 months (2-year OS 51.5%). Severe acute toxicity (≥G3, 34.0%) consisted mainly of G3 dysphagia during radiotherapy (24.0%). Severe late toxicity (≥G3) was observed in 9 patients (6.7%).

Conclusion: Individualized dose prescription in concurrent chemo-radiation based on normal tissue constraints is feasible, even in patients with large tumor volumes and multi-level N2-3 disease, with acceptable severe late toxicity and promising 2-year survival.