Title: Chloroquine with (radio-)chemotherapy in small-cell lung cancer: a report of 2 phase I trials.

Authors: B.J.T. Reymen MD[1], K.M.A. Rouschop PhD[1], L. Hendriks MD PhD[2], A.M.C. Dingemans MD PhD[2,3], M. Hochstenbag MD PhD[2], D. De Ruysscher MD PhD[1], P. Lambin MD PhD[4].

Affiliations:

[1] Department of Radiation Oncology (MAASTRO), GROW, School for Oncology and Developmental Biology, Maastricht University Medical Centre+, Maastricht, The Netherlands.

[2] Department of Pulmonology, Maastricht University Medical Centre and School for Oncology and Developmental Biology, University Maastricht, Maastricht, The Netherlands

[3] Dept of Pulmonology, Erasmus Medical Center, Rotterdam, The Netherlands

[4] Department of Radiation Oncology (MAASTRO, The D-Lab), GROW-School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, the Netherlands

Corresponding author:

Bart Reymen, Dr Tanslaan 12, 6229 ET Maastricht, The Netherlands.

E-mail address: bart.reymen@maastro.nl

Telephone: 0031884455600

Abstract:

Background: Small cell lung cancer (SCLC) treatment yields poor results. Chloroquine can block autophagy, thus enhancing SCLC but also normal cell response to chemotherapy and radiotherapy.

Methods: We conducted two phase I trials: in NCT00969306 stage IV SCLC patients received chloroquine daily during 5 cycles of cisplatin-etoposide chemotherapy. In NCT01575782 stage III SCLC patients received 4 cycles of cisplatin-etoposide concurrent with thoracic irradiation (30 fractions of 1.5 Gy, twice daily), with chloroquine only during radiotherapy.

Results: Inclusion halted for slow accrual after inclusion of 3 stage IV and 5 stage III patients at the first dose level (100 mg chloroquine). Two patients experienced grade 5 (lethal) and 7/8 showed ≥ grade 3 hematologic toxicity. Three stage III patients (60%) developed grade 3 esophagitis.

Conclusion: in 8 patients treated with chloroquine combined with chemotherapy and radiotherapy hematologic and esophageal toxicity was most frequent. Definitive conclusions about safety cannot be drawn.

Trial registration: NCT00969306 (Reg. date 1-9-2009) and NCT01575782 (Reg. date 11-4-2012)

Keywords

Chloroquine, autophagy, SCLC, chemotherapy, radiotherapy, phase I trial

1. Background

Tumour hypoxia negatively influences treatment outcome in many solid tumours, including small cell lung cancer (SCLC). Hypoxic cells are more radio-resistant, more chemo-resistant and more prone to developing distant metastases than normoxic cells.[1]

A survival mechanism used by tumour cells during metabolic stress (e.g. hypoxia) is (macro-) autophagy. This process involves the formation of autophagosomes: double membraned vesicles which engulf cellular constituents and fuse with lysosomes to digest cytoplasmic content, proteins and organelles. These components are then recycled as nutrients.[2,3]

Chloroquine is an old anti-malarial drug known to block autophagy by inhibiting the lysosome-autophagosome fusion. Chloroquine has been shown to decrease the hypoxic fraction in tumours and enhance tumour responses to radiotherapy and chemotherapy. [2,4,5]

Despite high initial response rates to chemotherapy and radiotherapy, most patients with SCLC relapse quickly and die of progressive disease. SCLC has been demonstrated to exhibit hypoxia in a large proportion of lesions. [6] Thus, chloroquine might represent a cheap and innovative way to sensitize SCLC tumours to treatment. Because autophagy also occurs in normal tissues such as bone marrow, the combination with chloroquine could however increase treatment related toxicity. [7,8]

We conducted two phase I trials aimed at determining the maximally tolerated dose (MTD) of chloroquine when combined with standard chemotherapy or concurrent chemotherapy and radiotherapy for SCLC.

2. Materials and methods

2.1 Patient characteristics and study design

Patients with histologically proven SCLC stage IV amenable for first-line palliative chemotherapy with cisplatin-etoposide (PE) were eligible for inclusion in trial NCT00969306. Patients with SCLC stage I-III fit for treatment with concurrent chemo-radiation were eligible for trial NCT01575782. The most important inclusion criteria were: World Health Organisation performance status (WHO-PS) 0-2, adequate renal, hepatic, lung and bone marrow function, no recent severe cardiac disease, no cardiac arrhythmia (except atrial fibrillation controlled on medication), no cardiac conduction disturbances (or medication potentially causing them). A complete list of the inclusion criteria is provided at clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT00969306 and https://clinicaltrials.gov/ct2/show/NCT01575782).

2.2 S*tudy design*

Initially we designed 1 trial (NCT00969306) with 2 parallel arms, testing a dose-level of chloroquine in a cohort of patients treated with chemoradiation for stage I-III SCLC only after it had been proven safe in combination with chemotherapy in stage IV SCLC patients.

Due to the slow accrual of stage IV SCLC patients (see below) and following the advice of the METC, the original trial split into two separate trials, with trial NCT00969306 continuing for stage IV patients and trial NCT01575782 for stage I-III opening in April 2012.

Both trials were single arm, non-randomized phase 1 combination trials designed to determine the MTD of chloroquine when combined with standard treatment of SCLC. DLT was defined as predefined severe (≥G3) toxicity occurring within 6 weeks after the last chemotherapy administration (see figure 1). The MTD was defined as the dose of chloroquine administered in the last cohort without toxicity surpassing these predefined limits. In trial NCT00969306 eligible patients with stage IV SCLC were to receive chemotherapy consisting of cisplatin (75 mg/m²) on day 1 and etoposide (120 mg/m²) day 1-3 for 5 cycles (cycle duration 21 days). Patients were instructed to take chloroquine daily for the total duration of chemotherapy, i.e. including the days on which no chemotherapy was administered, until day 3 of the last cycle. In trial NCT01575782 patients took chloroquine as a radiosensitizer, for the duration of 3 weeks during chest radiotherapy on the primary tumour and the involved lymph nodes to a standard dose of 45 Gy, delivered in 30 twice-daily fractions in 3 weeks. Chemotherapy (PE) was identical to the other trial in stage IV disease, but comprised 4 cycles instead of 5. Radiotherapy was started as soon as logistically feasible after the initiation of chemotherapy (median time between start of chemotherapy and radiotherapy: 20 days (range: 15-44 days). Apart from regular blood tests during chemotherapy, patients underwent electrocardiograms, ophthalmologic and hearing tests at baseline and at the follow-up visit post-chemotherapy. For stage IV patients extra electrocardiograms, ophthalmologic and hearing tests were performed at d1 of cycle 3. Pharmacokinetics measurements and collection of circulating tumor cells were also initially included in the initial protocol, but after refusal of several patients during the first six months of the trial to participate partly because of these extra blood tests, an amendment was submitted and accepted to proceed without these tests.

Both trials were designed using a 3+3 design, escalating chloroquine in four dose-levels, each containing a minimum of 3 and a maximum of 6 patients. The starting dose of 100 mg was chosen because of potential cisplatin-induced renal impairment.

Treatment related toxicity was evaluated according to the NCI Common Terminology Criteria for Adverse Events (CTC AE) version 4.0 from start of chemotherapy until 6 weeks post-chemotherapy.

3. Results

In both trials, one of the first 3 patients in the first cohort (100 mg chloroquine) developed DLT (neutropenic sepsis grade V and thrombopenia grade V). Therefore in both trials the first cohort was expanded to 6 patients. Both trials were closed prior to completion of the expanded cohort due to slow accrual. Between July 2011 and September 2017, 3 patients with stage IV and 5 patients with stage III SCLC were included.

3.1 Reasons for slow accrual

In practice a large proportion of patients with stage IV SCLC did not fulfill the inclusion criteria due to a rapidly declining performance status at diagnosis, also urging physicians and patients to quickly engage chemotherapy. In several of the regional hospitals, chemotherapy is started no later than 1 day after pathological confirmation of SCLC, in some cases even regardless of completeness of staging. The extra days needed for consideration of trial participation and the time needed for extra tests (ocular examination, hearing tests) before trial treatment initiation held back eligible patients from participating as well as physicians from surrounding hospitals from referring patients to the academic hospital (Maastricht University Medical Centre) running the trial, which treats an average of 20 patients with stage IV SCLC each year.

Secondly, the logistics of this trial proved too difficult. In the initial versions of the protocol several extra blood samples were included for pharmacokinetics of both chloroquine and cisplatin, both at baseline and at the time of each chemotherapy cycle. Also blood samples were to be sent to a biobank for measurement of circulating tumor cells. Several patients

refused to participate due to the amount of tests described in the protocol, which led to an amendment (see Methods).

Lastly, in both trials 1 patient of the first cohort died, which prompted toxicity analyses, temporary interruption of patient inclusion and extension of the cohorts. To ensure transparency and optimize trial logistics, we decided to follow the advice of the METC to split the trials, which also interrupted inclusion. Each long term interruption led to a run-in period during which logistic processes had to be reinstalled and trial staff had to be reinstructed, which also hampered a fast restart of inclusion.

3.2 Toxicity

All AE observed in the included patients during the observation are summarized in table 1. Ophtalmologic examinations did not reveal any ocular toxicity.

Trial NCT00969306 (stage IV SCLC)

Two out of 3 patients developed grade 3 hyponatremia. Both had a syndrome of inappropriate antidiuretic hormone secretion (SIADH) at diagnosis for which fluid restriction and demeclocyclin in both and (briefly) tolvaptan in one patient was started. Both patients developed grade 2 renal insufficiency and were switched from cisplatin to carboplatin during the course of treatment. In all 3 patients hematologic toxicity occurred: anemia grade 3 developed in 2 and neutropenia grade 4 in all 3 patients.

One patient died during chemotherapy. This patient had grade 1 thrombopenia at inclusion, evolving to grade 2 at one day prior to the first cycle of chemotherapy. After the first cycle of

chemotherapy the patient developed pancytopenia and while lymphocyte count returned to normal, anemia and thrombopenia persisted at grade 1. A CT-scan after the second cycle showed disease progression. The third cycle of chemotherapy was canceled and chloroquine was stopped after 9 weeks of intake. Despite cessation of chloroquine, thrombopenia and anemia progressively worsened while blood tests showed a progressively rising level of erythroblasts that had been present from diagnosis. The patient eventually received palliative sedation because of severe hemoptysis and subsequently died two months after ending chemotherapy. Autopsy was not performed.

Trial NCT01575782 (stage I-III SCLC)

All patients completed radiotherapy. Of the 5 patients (all stage III disease), 1 developed acute neutropenic sepsis shortly after completing the fourth cycle of chemotherapy and died immediately after admittance to the emergency room. A pulmonary focus was presumed since he had already been admitted to hospital due to pneumonia after the third cycle. Ionic disturbances (grade 3 hypokalemia and hypernatremia) were also seen at admission. A second patient was also admitted for neutropenic fever, dysphagia grade 3, nausea and vomiting after the third cycle. Hypokalemia grade 4 developed in this patient after a 2L infusion of NaCl 0.9% over 24 hours without addition of potassium, which was corrected afterwards. One patient developed grade 3 hearing loss for high frequencies.

4. Discussion

We conducted these phase I trials to assess the tolerance of chloroquine as an autophagy inhibiting agent combined with cisplatin-etoposide chemotherapy and radiotherapy. In the 8 patients in the two studies combined, two possibly treatment related deaths were recorded. The patient with stage IV SCLC died under both persistent thrombopenia and progressive disease under chemotherapy. Although reported as rare, the thrombopenia may have been caused by chloroquine itself. Since autophagy has been reported to occur in normal tissues as a response to cisplatin and is involved in normal hematopoiesis and stem cell differentiation, chloroquine may also have enhanced chemotherapy induced bone marrow suppression. However, the continuous decline of the platelet count in the pre-treatment phase, rising erythroblast count and documented progression in several other organ systems are indirect indications of bone marrow invasion. [9,10] It must be mentioned that this patient fulfilled all criteria at inclusion, but the grade 2 thrombopenia which developed before treatment start should have been a reason to exclude him from participation. This was unfortunately not recognized as such by the trial staff at the time. The second patient had stage III SCLC and died from acute neutropenic sepsis shortly after completing chemo-radiation, which is a known risk when using PE chemotherapy: some series report up to 10 % treatment related deaths with PE chemotherapy, mostly due to neutropenic infection and septic shock.[11] Combining the data of the 8 patients treated in both trials however a high frequency of severe hematologic toxicity stands out. Neutropenia grade ≥3 occurred in 87% of patients, while in literature 40% up to 75% has been reported. [12,13] In our stage III patients the maximal neutropenia always occurred

after the end of chloroquine treatment, therefore a relationship between ingestion of chloroquine and maximal neutropenia can also not be excluded in these patients.
Patients receiving chloroquine only during radiotherapy did not experience more radiotherapy-related side-effects than expected. Grade 3 esophagitis developed in 3 out of 5 patients (60%), which is high in comparison to the 20-25% commonly reported in accelerated chemo-radiotherapy, but lasted for more than 6 weeks in none.[14,15] The high frequency hearing loss found in 1 patient (12%) is not uncommon, since the FDA reports ototoxicity in up to 31% of patients after receipt of only one dose of cisplatin 50 mg/m^2.

In conclusion, these trials testing the safety of chloroquine as an autophagy inhibitor combined with standard cisplatin-etoposide chemotherapy and radiotherapy for SCLC were not completed. Definitive conclusions about safety cannot be drawn, but our results suggest caution with respect to potential hematologic toxicity and acute esophagitis.

5. Declarations

5.1 Ethics approval and consent to participate

Both trial protocols were written in accordance with the declaration of Helsinki and approved by the Medical Ethical Committee of MUMC+ (protocol numbers NL40391.068.12 and NL 29321.068.09). All patients provided informed consent prior to inclusion.

5.2 Consent for publication: not applicable.

5.3 Availability of data and materials: all data generated or analysed during this study are included in this published article.

5.4 Competing interests: PL is a member of the advisory board of DualTpharma which received an orphan drug designation from EMA and FDA for chloroquine in Glioblastoma. All other authors declare to have no conflicts of interest.

5.5 Funding: Authors acknowledge the financial support provided from ERC advanced grant (ERC-ADG-2015, n° 694812 - Hypoximmuno) and by the Dutch technology Foundation STW (grant n° 10696 DuCAT & n° P14-19 Radiomics STRaTegy), which is the applied science division of NWO, and the Technology Program of the Ministry of Economic Affairs. Authors also acknowledge financial support from EU 7th framework program (METOXIA – n° 2008-222741, ARTFORCE - n° 257144 and ImmunoSABR - n° 733008), SME Phase 2 (EU proposal 673780 – RAIL), Kankeronderzoeksfonds Limburg from the Health Foundation Limburg, the Belgian cancer foundation and the Dutch Cancer Society (KWF UM 2011-5020, KWF UM 2009-4454, KWF MAC 2013-6425, KWF MAC 2013-6089, KWF 2015-7635).

Role of the funding sources: financial support only. None of the funding bodies had a role in the study design; in the collection, analysis and interpretation of data; in the writing of the manuscript nor in the decision to submit the article for publication.

5.6 Authors` contributions: BR, KR, AD, DdR and PL were involved in designing the clinical study. All authors contributed to the scientific discussions and interpretation of the results. BR wrote the manuscript with suggestions and contributions of all authors.

References:

1 Wilson, W. R. & Hay, M. P. Targeting hypoxia in cancer therapy. *Nature reviews. Cancer* **11**, 393-410, doi:10.1038/nrc3064 (2011).

2 Rouschop, K. M. *et al.* The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* **120**, 127-141, doi:10.1172/JCI40027 (2010).

3 Rouschop, K. M. *et al.* Autophagy is required during cycling hypoxia to lower production of reactive oxygen species. *Radiother Oncol* **92**, 411-416, doi:10.1016/j.radonc.2009.06.029 (2009).

4 Sotelo, J., Briceno, E. & Lopez-Gonzalez, M. A. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* **144**, 337-343, doi:144/5/337 [pii] (2006).

5 Jutten, B. *et al.* EGFRvIII expression triggers a metabolic dependency and therapeutic vulnerability sensitive to autophagy inhibition. *Autophagy* **14**, 283-295, doi:10.1080/15548627.2017.1409926 (2018).

6 Chapman, J. D. Measurement of tumor hypoxia by invasive and non-invasive procedures: a review of recent clinical studies. *Radiother Oncol* **20 Suppl 1**, 13-19 (1991).

7 Periyasamy-Thandavan, S. *et al.* Autophagy is cytoprotective during cisplatin injury of renal proximal tubular cells. *Kidney Int* **74**, 631-640, doi:ki2008214 [pii] 10.1038/ki.2008.214 (2008).

8 Quarless, S. A. Chloroquine enhances the cytotoxicity of cis-diamminedichloroplatinum II. *Cancer Biochem Biophys* **10**, 11-15 (1988).

9 Cao, Y. *et al.* Autophagy Sustains Hematopoiesis Through Targeting Notch. *Stem cells and development* **24**, 2660-2673, doi:10.1089/scd.2015.0176 (2015).

10 Rozman, S. *et al.* The generation of neutrophils in the bone marrow is controlled by autophagy. *Cell death and differentiation* **22**, 445-456, doi:10.1038/cdd.2014.169 (2015).

11 Berghmans, T. *et al.* A Phase III Randomized Study Comparing a Chemotherapy with Cisplatin and Etoposide to a Etoposide Regimen without Cisplatin for Patients with Extensive Small-Cell Lung Cancer. *Frontiers in oncology* **7**, 217, doi:10.3389/fonc.2017.00217 (2017).

12 Eckardt, J. R. *et al.* Open-label, multicenter, randomized, phase III study comparing oral topotecan/cisplatin versus etoposide/cisplatin as treatment for chemotherapy-naive patients with extensive-disease small-cell lung cancer. *J Clin Oncol* **24**, 2044-2051, doi:10.1200/JCO.2005.03.3332 (2006).

13 Belani, C. P. *et al.* Randomized phase III trial comparing cisplatin-etoposide to carboplatin-paclitaxel in advanced or metastatic non-small cell lung cancer. *Ann Oncol* **16**, 1069-1075, doi:10.1093/annonc/mdi216 (2005).

14 Turrisi, A. T., 3rd *et al.* Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. *N Engl J Med* **340**, 265-271 (1999).

15 Reymen, B. *et al.* Total gross tumor volume is an independent prognostic factor in patients treated with selective nodal irradiation for stage I to III small cell lung cancer. *Int J Radiat Oncol Biol Phys* **85**, 1319-1324, doi:10.1016/j.ijrobp.2012.10.003 (2013).

Tables and figures

Figure 1: Trial flowchart with dose limiting toxicity (DLT) and cohort expansion rules. (MTD= Maximally Tolerated Dose)

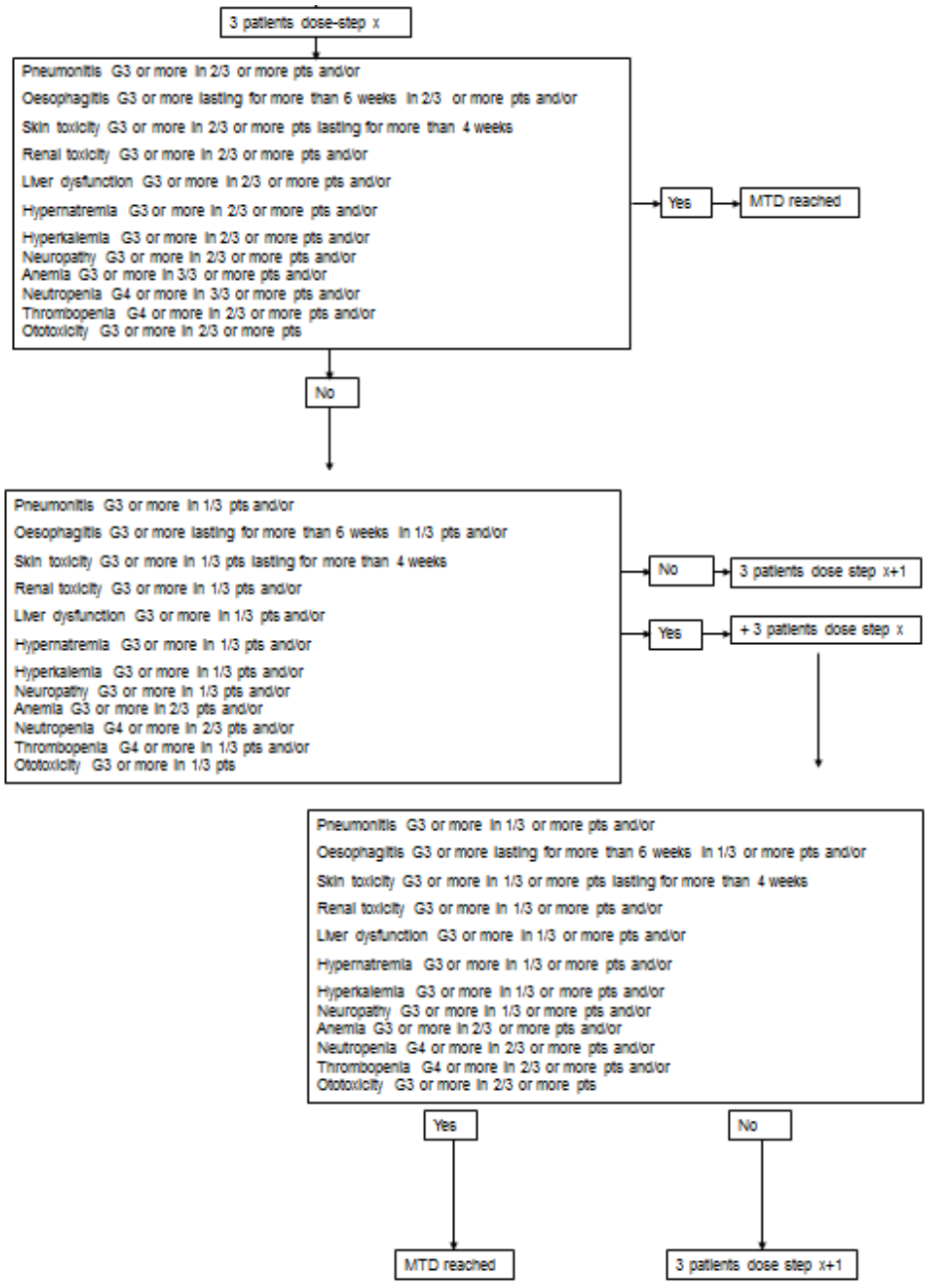

Table 1: Toxicity recorded per patient according to CTCAE 4.0 (Stage III: n=5, Stage IV: n=3).

STAGE/ptnr	III/1	III/2	III/3	III/4	III/5	IV/1	IV/2	IV/3
LAB: non-hematology								
Hypernatremia	0	0	3	0	0	0	0	0
Hyponatremia	0	0	1	0	1	0	3	3
Hyperkalemia	1	0	1	0	0	1	0	0
Hypokalemia	0	0	3	0	4	0	0	0
Hepatic function	1	0	1	0	1	1	0	0
Renal function	0	0	0	0	2	2	2	1
LAB: hematology								
Anemia	2	1	3	2	3	3	3	1
Neutropenia	3	1	5	3	4	4	4	4
Thrombopenia	1	0	0	0	4	1	5	0
OTHER								
Ocular toxicity	0	0	NA	0	0	0	NA	0
Fatigue	1	1	3	1	1	2	2	0
Fever	0	0	0	3	1	0	0	0
Headache	0	0	0	1	1	0	0	0
Nausea	1	1	2	2	3	0	0	0
Weight loss	0	0	0	0	2	1	2	1
Dehydration	0	0	0	0	3	0	0	0
Abdominal pain	1	0	0	0	0	0	0	0
Dysphagia	0	3	3	2	3	0	0	0
Dyspepsia	1	1	0	0	1	0	0	0
Cough	1	1	1	0	1	0	0	0
Dyspnea	1	1	2	1	1	0	0	0
Atrial fibrillation	0	0	0	0	3	0	NA	0
QT-elongation	0	0	0	0	1	0	NA	0
Neuropathy	0	0	0	0	0	0	0	0
Ototoxicity	2	0	NA	3	0	1	NA	0

Legend table 1: toxicity recorded for each included patient separately, scored according to CTCAE 4.0. Patients included in trial NCT0157578 containing only stage III patients treated with chemoradiation have prefix III followed by inclusion number (eg. III/1). Stage IV patients (trial NCT00969306) treated with chemotherapy have prefix IV followed by inclusion number.