ARTFORCE FINAL REPORT

Executive summary

The ARTFORCE project succeeded by introducing very sophisticated imaging and radiotherapy tools into daily clinical practice to improve treatment outcome of patients with advanced Head & Neck and Lung tumours. Within interlinked projects treatment-specific tumour response predictors were developed for selection of the optimal treatment regimen for the combination of drugs and irradiation.

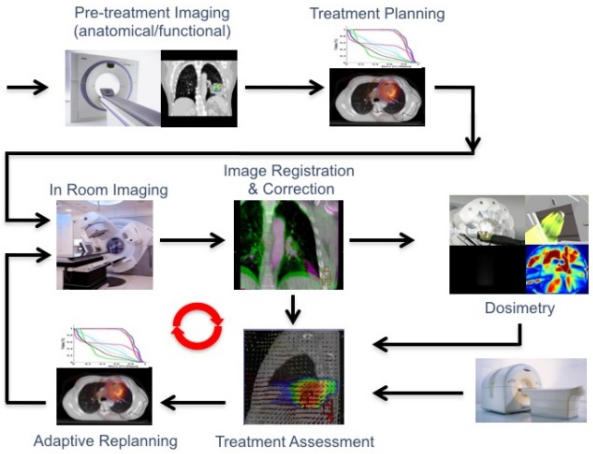

Figure 1:The process of adaptive intensity modulated image guided radiotherapy (IGART).

As a first step patient tailored irradiation was introduced with adapted intensity modulated image guided radiotherapy (IGART), based upon CT, MRI and PET imaging before and during treatment. For this precise delivery of the radiation dose novel on line image guided adaptive irradiation techniques were developed, including very advanced quality assurance methods with daily cone beam CT imaging and in vivo dosimetry. This created the possibility of treatment adaption based upon the response of the tumour. This IGART allowed delivery of higher tumour radiation doses with sparing of the normal tissues. By targeting on the most radioresistant part of the tumour with IGART it aimed at higher tumour control rates, with less side effects and therefore improved quality of life (Figure 1.)
These methods were tested and approved within two major clinical trials carried out in the ARFORCE consortium consisted of 12 university hospitals in 7 European countries:

- A randomised Phase III trial with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer
- Dose-escalation by boosting radiation dose within the primary tumour on the basis of a pre-treatment FDG-PET-CT scan in stage IB, II and III NSCLC: A randomized phase II trial

Withholding ineffective, toxic treatments and to decrease community costs by targeting expensive treatments to those who will benefit was another objective of this project. The combined modality

treatment with radiotherapy and Cetuximab for Head & Neck tumours appeared to be less effective than the combination of radiotherapy and Cisplatinum even for patients with patients with known HPV/p16 status. Therefor treatment-specific tumour response predictors were developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during the treatment. Further exploration of biomarkers, revealed novel predictors and prognostic biomarkers, including in a multi-centric setting tested innovative imaging modalities with radiomics. Within this project proof of concept was provided that molecular information can be derived from standard medical images with radiomics and it provided prognostic for patients with Head & Neck cancer. The validation of these biomarkers for predicting the Cisplatinum and radiation sensitivity will be performed after closing the ARTFORCE Head & Neck Phase III clinical trial in 2019.

The improvement of the overall level of radiation oncology in Europe by introducing and validating in routine clinical practice the methods for fully-controlled image guided adapted radiotherapy was one of the main objectives. Therefor several by presentations were given at courses and meetings for the European Radiotherapy community: (ESTRO). As a result, the developed methods are now introduced in daily clinical practice in several hospitals. This was accompanied with the publication of newsletters and 222 peer reviewed papers on this ARTFORCE project

Summary description of project context and objectives

Background

Surgery, radiotherapy and chemo-radiation, i.e. the combination of radiotherapy with chemotherapeutics such as Cisplatinum or Cetuximab, are treatment options for patients suffering from Head & Neck cancer or Lung cancer. However considerable proportion of patients is diagnosed at a late stage and is treated with a combination of radiotherapy and cisplatin. This combination is effective, although not all patients benefit and less than half of the patients will be cured. By introducing new image guided targeted radiotherapy regimen higher radiation doses to the tumour, while sparing the surrounding normal tissues will lead to higher cures rates without increasing the side effects. To prevent that patients suffer from severe side effects without benefiting from the treatment, specific and sensitive biomarkers are urgently needed to support treatment decision. Withholding ineffective, toxic treatments and while targeting expensive treatments to those who will benefit will lead to improved quality of life and decreased community costs. To this end, parallel to the novel irradiation and quality assurance programs, treatment-specific tumour response predictors were developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during the treatment.

Objectives

The aim of this project was to improve treatment outcome more specifically in advanced Head & Neck and non-small cell lung cancer patients by:

- Redistributing the radiation dose with adaptive image guided and intensity modulated radiotherapy towards the most resistant part of the tumour.
- Introducing novel 3-dimensional (3-D) optimized image guided radiotherapy techniques and quality assurance programs.
- Developing treatment-specific tumour response predictors, biomarkers and radiomics developed for individualizing patient's treatment,
- Disseminate the results to the community by means of exposure through scientific community and development of general applicable software and tools in combination with commercial partners.

Description of work performed and main results

The ARTFORCE project succeeded by introducing very sophisticated imaging and radiotherapy tools into daily clinical practice to improve treatment outcome of patients with advanced Head & Neck and lung tumours. The work carried out in this project aimed at higher tumour control rates with improved quality of life, by using MRI and PET imaging for targeting on the most radioresistant part of the tumour. This was achieved by enabling tailored irradiation to the most active parts of the tumour with the adapted image intensity modulated guided radiotherapy (IGART) based upon CT, MRI and PET imaging before and during treatment.

For precise delivery of the radiation dose novel on line image guided adaptive irradiation techniques were developed and very advanced quality assurance methods with daily CT cone beam imaging and in vivo dosimetry.

This allowed delivery of higher tumour radiation doses with sparing of the normal tissues. These methods were tested and approved within two major clinical trials:

- A randomised Phase III trial with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer
- Dose-escalation by boosting radiation dose within the primary tumour on the basis of a pre-treatment FDG-PET-CT scan in stage IB, II and III NSCLC: A randomized phase II trial

These clinical trials were carried out in the ARFORCE consortium, consisted of 9 university hospitals in 7 European countries: Christie NHS Foundation Trust: Maastro clinics, University Medical Center Utrecht; University Medical Center Groningen; Erasmus Medical Center; Institute Catala de la Salut, Vall d'Hebron; Gustave Roussy Cancer Institute; Karolinska Institute; Netherlands Cancer Institute-Antoni van Leeuwenhoek, and 3 associated university hospitals Academic Medical Center Amsterdam, University hospital Leuven, Rigshospitalet University Hospital Copenhagen

Withholding ineffective, toxic treatments and to decrease community costs by targeting expensive treatments to those who will benefit was another objective of this project. We showed that the combined modality treatment with radiotherapy and Cetuximab for Head & Neck tumours was less effective than the combination of radiotherapy and Cisplatinum even for patients with patients with known HPV/p16 status. Therefor treatment-specific tumour response predictors were developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as, well as functional and anatomical imaging predictors early during the treatment.

Within the ARTFORCE project there are several interlinked work packages.
Work package 2 (*Adaptive Radiotherapy to account for anatomical changes*) resulted in implementation of adaptive re-planning in all centres participating in the head-and-neck trial. Methods to quantify the accuracy of deformable image registration algorithms were developed. Furthermore, novel methods to adapt treatments together with a decision rule to select patients for adaptive replanning were evaluated.

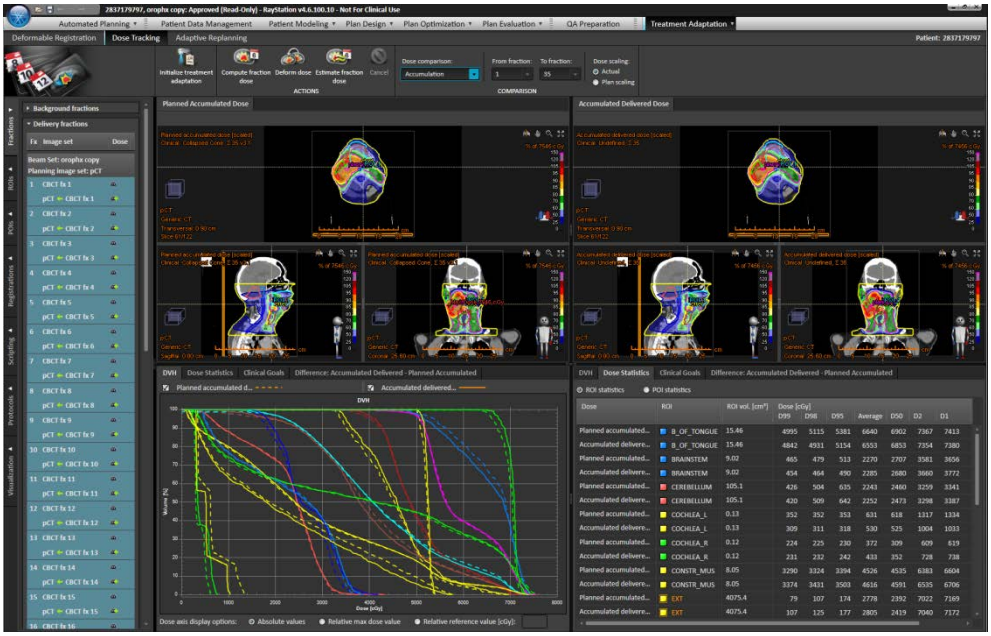

Figure 2: Screenshot of the dose accumulation graphical user interface comparing the planned and accumulated dose distribution

In work package 3 (*Biological adaptive treatment planning in the presence of advanced techniques*) algorithms were developed for biologically optimized treatment planning aimed at eradication of the tumour with minimal normal tissue morbidity based upon interim PET scans and recurrence pattern in the clinical trials. Also, implementation was achieved of the algorithm as an add-on module into a treatment planning system intended to estimate accumulated dose and patient specific treatment response making it possible to replan and eventually biologically adapt the treatment based on this additional information.

Work package 4 (Three dimensional in-vivo dosimetry) implemented and validated three dimensional in-vivo to allow early on-line detection of errors in treatment delivery of sophisticated radiotherapy in each participating centre. It a calibrated on-board flat panel electronic portal imaging device to act as a two dimensional dosimeter to capture the actual radiation delivered during the treatment. Additionally it uses the on-board cone-beam CT scanner to capture the anatomy at the time of treatment. These were combined into a QA platform to make a comprehensive verification platform that works for all major linear accelerator vendors.

Work package 5 (Biological *markers to predict the response of Head & Neck tumours to Cetuximab or Cisplatinum + Radiotherapy*) provided important prognostic and predictive information. A meta-analysis revealed prognostic impact of the immune infiltrate: novel organ-specific features of the immune infiltrate in distinct cancer types, as well as a strategy for defining new prognostic biomarkers. The calreticulin expression constitutes a new powerful prognostic biomarker that reflects enhanced local antitumour immune responses in the lung. While the importance of formyl peptide receptor 1 mutation (FPR1) was highlighted in chemotherapy-induced anticancer immune responses. It was shown that overexpression and hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1) and the downregulation of pyridoxal kinase (PDXK), correlated with elevated apoptosis resistance. Further exploration showed that PAR and PDXK were predictive biomarkers in non-small cell lung cancer: For Head & Neck cancer radiomics features provided an added value to HPV status as prognostic and predictive biomarker treated with the combined modality radiotherapy with Cisplatinum or Cetuximab (Figure 3).

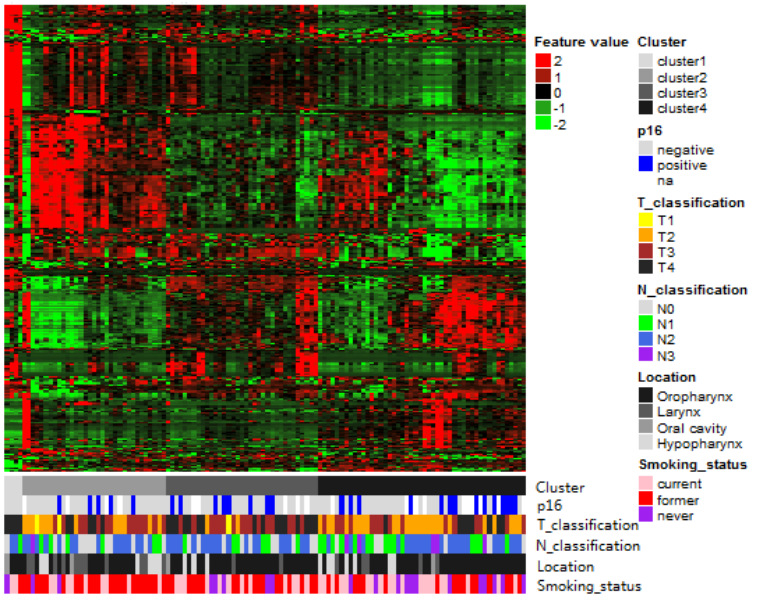

Figure 3. Heatmap displaying Z-scores of the feature values for the 544 radiomics features for the whole cohort (N = 120). Unsupervised clustering revealed 4 clusters of patients with similar radiomics expression patterns. We compared the 4 clusters of patients with clinical parameters, and found significant correlation with T classification (P = 3.3 * 10-5), primary tumour location (P = 0.01) and a nonsignificant correlation with smoking status (P = 0.09), whereas no correlation was observed with p16 status (P = 0.19) and N classification (P = 0.22).

The combination of microvascular density and CA-IX expression might give additional prognostic information in these patients with known HPV status. High CD8+ TIL level was an independent prognostic factor independent of HPV/p16status. CD8+ TILs and PD-L1 expression could provide complementary information to HPV status in selecting subpopulation for treatment de-intensification. Intraepithelial macrophage expression may play different roles in patients with p16+ vs. p16- disease. CD163+ cells density in stroma may provide information for selecting suitable patients for concurrent Cetuximab or Cisplatinum with radiotherapy. Established molecular signatures were assessed for their

response prediction value in HNSCC patients treated with Cisplatinum and radiotherapy. Drug response and DNA repair defect linked expression markers were therefore developed and further improve the detection of poor prognosis patients. The validation of these biomarkers for predicting the Cisplatinum and radiation sensitivity will be performed after closing the ARTFORCE Head & Neck Phase III clinical trial in 2019.

Work package 6 (*Standardisation and innovative molecular imaging for prediction and decision making*), The main objective of the work package was to asses, in a multi-centric setting, innovative imaging modalities allowing better predicting outcome and individualizing. We investigated the possibilities of imaging of hypoxia and the presence of EGFR in patients with PET and DCE-CT. Predictive value of HX4 PET and DCE-CT imaging (Figure 4). A combined analysis of the patients in the PET-boost trial and another trial in non-small cell lung cancer with the same pre-treatment imaging protocol (NCT01210378), showed that HX4 PET is a prognostic marker for overall survival.

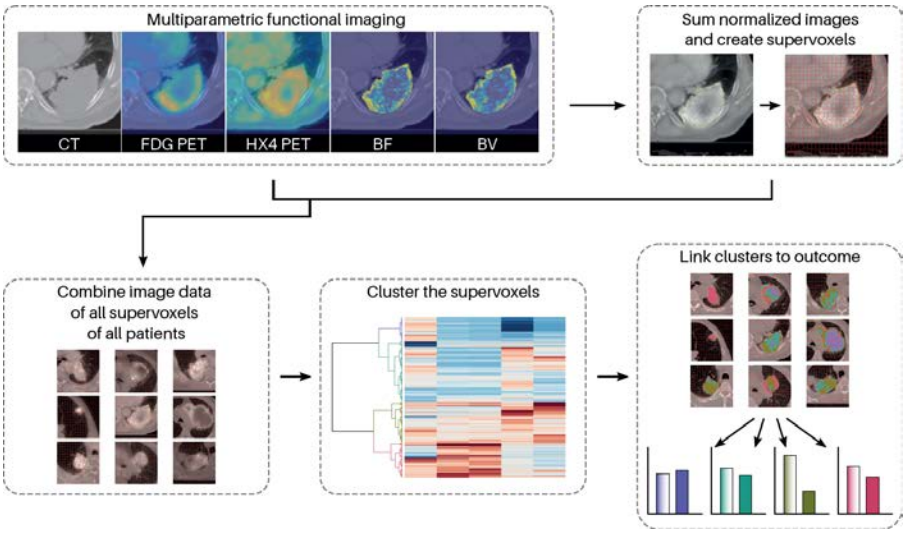

Figure 4. The workflow of the data analysis:

Also, radiomics was explored as a high-throughput mining of quantitative image features from standard-of‑care medical imaging that enables data to be extracted and applied within clinical-decision support systems to improve diagnostic, prognostic, and predictive accuracy. Our study provides proof of concept that molecular information can be derived from standard medical images and shows potential for radiomics as imaging biomarker of HPV status. The interchangeability was investigated of planning CT and conebeam CT (CBCT) extracted radiomic features. Furthermore, a previously described CT based prognostic radiomic signature for non-small cell lung cancer (NSCLC) patients using CBCT based features was validated. The previously developed radiomics signature has prognostic value for overall survival in three CBCT cohorts, showing the potential of CBCT radiomics to be used as prognostic imaging biomarker.

In work package 7 (*Dose-escalation by boosting radiation within primary tumour based on a pre-treatment FDG-PET-scan*): a randomized phase II clinical trial in locally advanced NSCLC: was carried out. Three extra centres have contributed patients to this trial, without formerly entering the consortium. An interim analysis on toxicity showed toxicity profiles in line with iso-toxic dose escalation. All patients received a follow-up FDG PET-CT scan, 3 months post treatment. These scans

reveal increased CT densities and PET-uptake in irradiated normal lung tissue compared to scans acquired prior to treatment. Subsequently, a dose effect relationship for density changes and PET uptake was investigated. Both imaging modalities demonstrate a linear dose effect relation between 15Gy and 60 Gy although the slope differed substantially between patients. Above 70 Gy the relationship plateaus indicating a saturation of the dose-effect relationship. Moreover, severe damage was rare peripherally in the lung. These relationships could be exploited in plan optimization. The first toxicity results of the PET-boost trial showed that individualized dose-escalation up to normal tissue constraints was feasible and not related with unexpected acute or late toxicity. Therefore, it was concluded that dose-escalated radiotherapy to the primary tumour or regions with high FDG-uptake within the primary tumour did not reveal an unexpected or excess of acute and late toxicity. The trial is now closed with 107 randomized and 150 registered patients. The final analysis for the end results will be performed in 2018, when all patients have at least one year follow up.

In work package 8: (*Increasing the therapeutic ratio for Head & Neck cancers by pre-treatment selection and dose redistribution*) the phase III ARTFORCE clinical trial: A randomised study with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer after initial delays is well underway it accrued already 181 patients and will finish in the beginning of 2019. In the first approved version of the protocol, the trial consisted of a 2x2 design with four treatment arms, comparing 1. Cisplatinum versus Cetuximab and 2. Standard radiation dose to a dose-redistribution based on the FDG-PET activity. Furthermore, a pre-treatment Zirconium Cetuximab scan was done to improve future patient selection and an additional PET scan in de second week of treatment to facilitate development of biological adaptive radiotherapy in WP3. Patients with locally advanced (at least T3-T4) tumours of the oropharynx, hypopharynx or oral cavity who are fit for treatment with concurrent chemotherapy can be included. In 2014, after inclusion of 17 patients, the treatment schedule was amended because the pharmaceutical company stopped the free provision of Cetuximab. Therefor the trial was changed to a two-arm study comparing standard radiotherapy to dose-redistributed radiotherapy, with in both arms a conventional fractionated radiotherapy scheme with 3-weekly cisplatin. The Zirconium scan was replaced by a radiomics task, as well as an optional hypoxia imaging task with HX4 PET scans. The Head & Neck trial will be finalized in the beginning of 2019. At that time we will start with analysing the data from the patients in the clinical trial and work package 5 with validation of the predictive assays.

WP 9 (*Distribution of knowledge and expertise*) took care of the dissemination of the results aiming at improvement of the overall level of radiation oncology in Europe. This was achieved by introducing and validating in routine clinical practice the methods for fully-controlled image guided adapted radiotherapy Therefor several by presentations were given at courses and meetings for the European Radiotherapy community (ESTRO). This was accompanied with the publication of newsletters and 222 peer reviewed papers on this ARTFORCE project

Description of the main S&T results/foregrounds

Summary of the major results

This ARTFORCE project aimed at improving the treatment outcome in patients with Head & Neck or lung cancer treated with a combined modality of radiotherapy and systemic treatment. The improvement in better tumour control will be reached by:
-delivering higher radiation doses to the tumour while minimizing the radiation dose to normal tissues
-by developing treatment-specific tumour response predictors, i.e. genetic predictors for Cisplatinum and radiation sensitivity, as well as functional and anatomical imaging predictors early during the treatment.
This has been obtained by:

1. Novel irradiation methods using information from innovative imaging approaches for the individual treatment design. (WP 2,3 and 6)
2. Adapting the radiation treatment plans to individual patient's anatomical and biological changes during treatment. (WP 2,3,7 and 8)
3. Designing and validating new QA methods for sophisticated high-tech radiation procedures. (WP 2,4,7 and 8)
4. Developing and validating methods of patient selection for treatment with a combination of radiation and Cisplatinum or Cetuximab. (WP 5, 6,7 and 8)

Several by presentations were given at courses and meetings for the European Radiotherapy community (ESTRO) in order to make the results available for routine use in daily clinical practice. This was accompanied with the publication of newsletters and 222 peer reviewed papers on this ARTFORCE project

Background

Surgery, radiotherapy and chemo-radiation, i.e. the combination of radiotherapy with chemotherapeutics such as Cisplatinum or Cetuximab, are treatment options for patients suffering from Head & Neck cancer or Lung cancer. A considerable proportion of patients is diagnosed at a late stage and is treated with a combination of radiotherapy and cisplatin. This combination is effective, although not all patients benefit and less than half of the patients will be cured. Higher cures rates without increasing the side effects will be possible by introducing new image guided targeted radiotherapy treatment regimens with higher radiation doses to the tumour, while sparing the surrounding normal tissues. To prevent that patients suffer from severe side effects without benefiting from the treatment, specific and sensitive biomarkers are urgently needed to support treatment decision. Withholding ineffective, toxic treatments and by targeting expensive treatments to those who will benefit will lead to improved quality of life and decreased community costs. To this end, parallel to the novel irradiation and quality assurance programs, treatment-specific tumour response predictors were developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during the treatment.

Objectives

The aim of this project is to improve treatment outcome more specifically in advanced Head & Neck and non-small cell lung cancer (NSCLC) patients by:

1*) Enlarging the therapeutic ratio between tumour control and normal tissue damage by redistributing the dose*. Ineffective locoregional control is the primary reason for relapse in Head & Neck cancer, as well as in non-small cell lung cancer. Optimizing local control is now becoming possible by redistributing the radiation dose, creating inhomogeneous dose distribution towards the most resistant

part of the tumour that has been defined by18F-fluoro-deoxyglucose-based positron emission tomography (FDG-PET) scan, instead of a conventional homogenous doses distribution with 1-2 cm margin around the tumour area.

2) *Ensuring 3-D quality control in vivo*; Quality assurance (QA) must be improved as new treatment techniques are developed. A New York Times article on radiation accidents used the heading "As Technology Surges, Radiation Safeguards Lag", to alert about the danger of introducing new techniques without adequate quality control. To assure accurate radiation delivery we will introduce novel 3-dimensional (3-D) optimized image guided radiotherapy (IGRT) techniques. 3-D monitoring and adapting of the radiation dose will be performed with repetitive anatomical Cone Beam CT scans on linear accelerators, as well as biological imaging by FDG-PET. Finally, 3-D in vivo portal dosimetry will be performed during the course of radiotherapy (RT) for optimal QA.

3) *Maximizing the benefit of combined modality treatment*. Both Cisplatinum and Cetuximab in combination with RT have resulted in the improvement of local control and survival. However, Cisplatinum is only effective in a fraction of patients, those with cisplatin-sensitive tumours. Moreover, the rather expensive Cetuximab is only useful for the treatment of tumours that incorporate high levels of this agent. Adequate selection of patients thus can be expected to reduce unnecessary treatments, thus minimizing toxicity and cost. For this, the uptake of Cetuximab will be measured with 89Zr labelled Cetuximab, while Cisplatinum sensitive tumours will be selected using adequate biomarkers (e.g. ERCC1 and MDS2) that can be assessed by immunohistochemistry. Therefore treatment-specific tumour response predictors were developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during the treatment.

4) *Disseminate the results to the community* by means of exposure through scientific community and development of general applicable software and tools in combination with commercial partners.

WP2 Adaptive Radiotherapy to account for anatomical changes

To develop adaptive radiotherapy techniques to optimally account for the temporarily variant patient model, therefor the accuracy of deformable image registration needs to be quantified for the purpose of automatic contour propagation. To accumulate the dose of the patients treated on the clinical trials, CBCT based dose recalculation and accurate than in-vivo dosimetry program needs to be developed. Also decision rules needs to be developed to select patients for adaptive replanning, including a database for dose accumulation.

Results

The experimental arm of the head-and-neck trial (Work package 8) includes PET based simultaneous integrated boost and a plan adaptation after 2 weeks of treatment. This treatment strategy was successfully implement and tested in all participating centers: Maastro clinics; University Medical Center Utrecht,; University Medical Center Groningen,; Erasmus Medical Center,; The Christie NHS Foundation Trust,; Institute Catala de la Salut, Vall d'Hebron, Gustave Roussy Cancer Institute,; Karolinska Institute; Netherlands Cancer Institute,

The accuracy of deformable image registration for the purpose of automatic contour propagation was quantified. To that end, the transitivity error was determined by propagating the contours over a circle of 3 different CT scans of 20 head-and-neck cancer patients. The propagated contours were subsequently compared to the original contours. It was shown for 2 independent image based deformable image registration algorithms that the accuracy (median of the 90% of shortest distances histogram) was below 2mm for most structures for both algorithms (see figure 5). Similarly, the accuracy of deformable registration for patient modelling and dose accumulation was quantified using

the residual displacement of implanted fiducial markers in involved lymph nodes of 12 locally advanced lung cancer patients.
The mean vector length of residual displacement was below 2mm (figure 6).

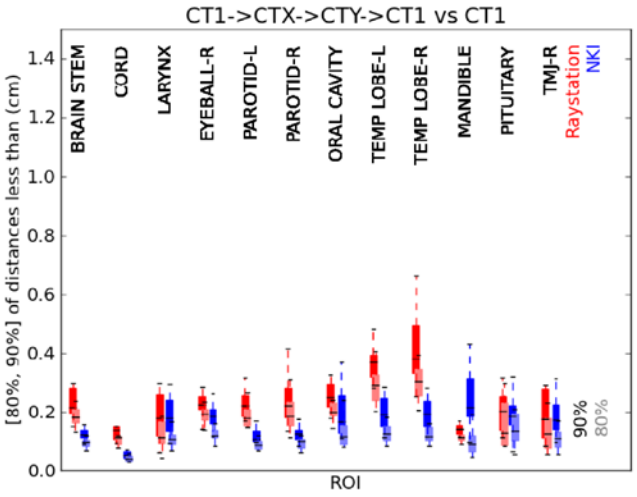

Figure 5: Comparing the original surfaces to those thrice-deformed by 2 different deformable registration algorithms. The distance which encompasses 90% or 80% of the shortest distance mappings characterizes the dissimilarity of the surfaces. The extent of the boxes indicates the upper and lower quartiles, while the whiskers indicate the maximum and minimum values.

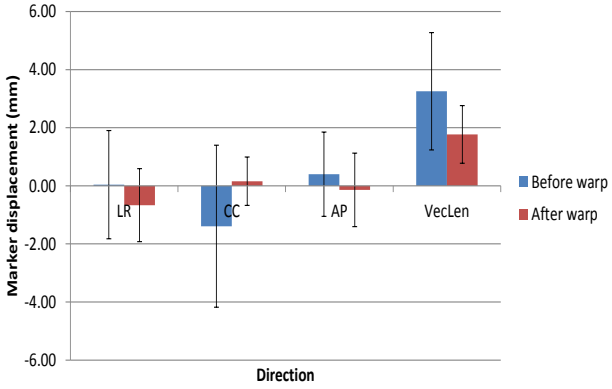

Figure 6: Mean residual displacement of the mediastinal lymph node markers before and after deformable Cone Beam CT to planning CT registration

In order to collect all relevant imaging and planning data for improved outcome modelling and data mining, the electronic case report form (eCRF) of both the head-and-neck trial and lung trial have been extended with data anonymization and upload functionality. A schematic outline of this infrastructure is depicted in Figure 6. The eCRF allows for the upload of all treatment planning scans, treatment plans, planned dose distributions, daily image guided scans, spatial registration objects, and follow-up scans. The upload servers and software are being installed in the participating centres. About 1/3 of the included patients in both trials have been uploaded to the central server and work continuous to complete these databases.

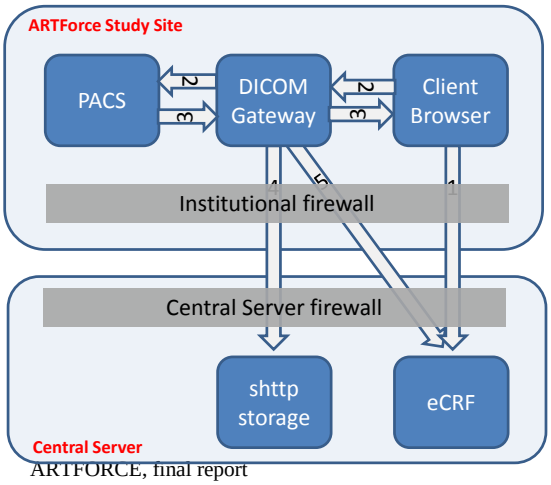

Figure 7: 1) Client browser selects the eCRF 'PACS objects for study subject' page from eCRF. 2) This page loads an aspx page from the DICOM gateway for selecting PACS objects for this patient. 3) The user enters a patient's hospital record number and receives a list of objects stored in PACS and selects the corresponding PACS object to be added to the patient eCRF. 4) DICOM gateway asynchronously sends selected objects to central storage over shttp. 5) DICOM Gateway sends the anonymized object references to the eCRF.

In order to accumulate the dose of the patients treated on the clinical trials, the dose first needs to be recalculated on the anatomy of the day. As many different treatment machines and dose calculation engines are being used in the participating hospitals and the corresponding beam fits are not available, an alternative method was developed and tested. Based on the available planned dose distribution and linac type, the treatment planned was mimicked in a single treatment planning system (RayStation) based on a similar treatment machine by optimizing the treatment plan with the planned dose distribution as target dose. This approach is accurate to within 1 percent which is well within the required accuracy for dose accumulation.

An infrastructure for automated dose accumulation was developed to facilitate efficient evaluation of the delivered dose. The main components are the establishment of CBCT values to density table using a bulk density approach, dose computation on CBCT by moving the beam setup according to the treatment position alignment and using the density table and deformation of the dose computed on CBCT in to pCT geometry. Average time for accumulation is 53 s for one fraction.

A novel adaptive strategy was developed to reduce systematic deformations by modifying the pCT to the average anatomy as observed in a repetitive imaging series during the initial fractions of radiotherapy. To that end, first deformable image registration was used to map each element of the repetitive imaging series on the planning CT. The resulting series of deformable vector fields (DVF) is averaged, inverted and applied to the planning CT to generate the average patient model (Figure 8). This method was tested on daily cone beam computed tomography (CBCT) scans of 25 head-and-neck cancer patients. Residual uncertainties were quantified relative to 1) the planning CT, 2) the average patient model based on the first 10 fractions, 3) the weekly average patient model. Relative to the unadapted reference, systematic deformations reduced by a single intervention by 20%-40%. Weekly adaptation allows for systematic deformation reduction of 35%-60%. Similar performance was achieved for locally advanced lung cancer patients.

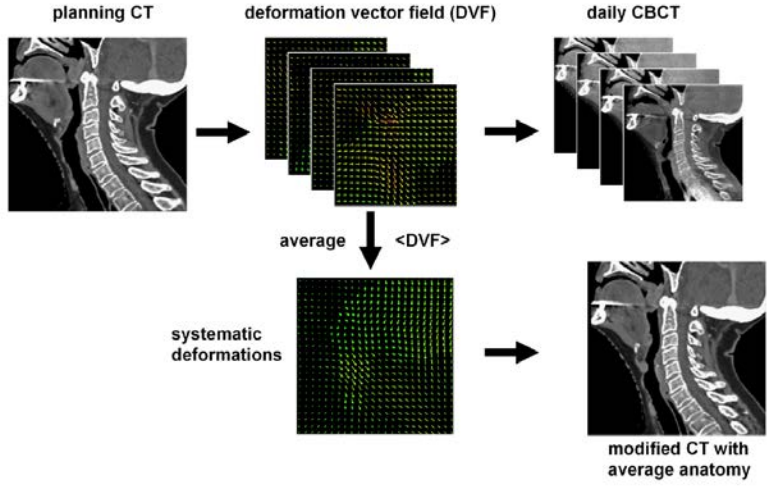

Figure 8: Schematic representation of the average patient anatomy model generation.

To balance workload and efficacy of adaptive replanning, a decision rule for adaptive radiotherapy was developed that selects patients with the largest dosimetric discrepancies. To that end, the daily

accumulated dose was calculated for 89 Head & Neck cancer patients and differences (ΔDVH-parameters) between planned and delivered were determined. Subsequently, given the first f fractions, regression analysis was applied to estimate the final accumulated ΔDVH-parameters (accumulated – planned) and the corresponding prediction-interval at uncertainty level a. Patients for which the prediction-interval was below\above (for CTV or OAR respectively) the c% highest\lowest final ΔDVH-parameter distribution were marked as candidates for adaptive intervention (Figure 9). Receiver-Operator-Curves were constructed by varying the uncertainty level a, simultaneously evaluating all OARs and CTVs. Patients candidate for adaptive replanning could be identified with an AUC ranging from 0.55 (fraction 5), 0.85 (fraction 12) to 0.95 (fraction 20), more or less independent of c. In conclusion, a decision rule based on daily dose accumulation was successfully developed to predict predefined deviations in DVH-parameters early in treatment with high accuracy. The clinical workload can be balanced with tolerance to dose discrepancies.

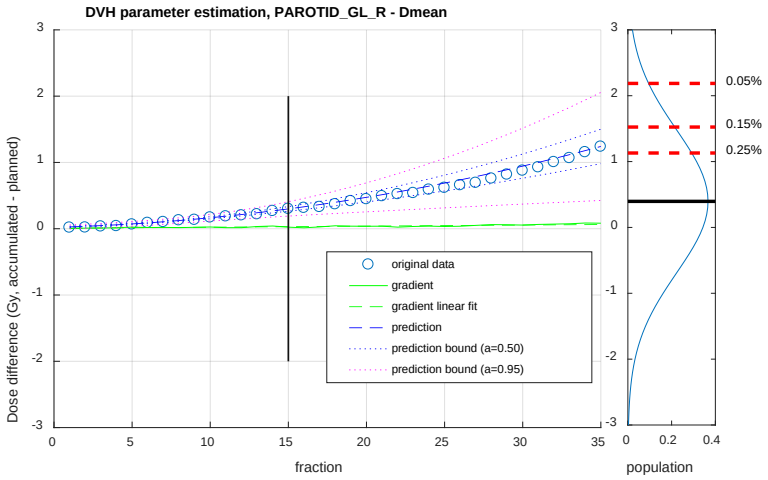

Figure 9: ΔDVH parameter prediction curve with boundary estimation (a=0.5% and 0.9%), example at fraction 15 (left), against population characteristics (right) with cut-off values at 0.95, 0.85 and 0.75.

WP3 Biological adaptive treatment planning in the presence of advanced techniques

The objectives of WP3 were the development of an algorithm for biologically optimized treatment planning aimed at eradication of the tumour with minimal normal tissue morbidity based upon interim PET scans and recurrence pattern in the clinical trials and the implementation of the algorithm as an add-on module into a treatment planning system intended to estimate accumulated dose and patient specific treatment response making it possible to replan and eventually biologically adapt the treatment based on this additional information.

The main results and highlights of WP3 are therefore well-aligned with the overall objectives. The results were achieved in a sequential manner starting from the assessment of the early tumour responsiveness and the corresponding effective radiosensitivity for individual NSCLC patients, based on two successive FDG-PET scans. In order to perform this assessment, an operational quantity, effective radiosensitivity, αeff, was theoretically introduced and practically determined for twenty-six

non-small cell lung cancer (NSCLC) patients previously treated with chemoradiotherapy at the Department of Radiation Oncology (MAASTRO clinic), Maastricht University Medical Center, The Netherlands. Fifteen patients underwent sequential chemoradiotherapy and 11 patients received concomitant chemoradiotherapy. Correlations were sought between two parameters derived from the distributions of the effective radiosensitivity determined at voxel level - average αeff or the fraction of negative αeff values - and the overall survival at 2 years. Separate analyses were performed for the primary GTV, the lymph node GTV and the CTVs. The results showed that the patients receiving sequential chemoradiotherapy could be divided into responders and non-responders, using a threshold for the average αeff of 0.003 Gy-1 in the primary GTV, with a sensitivity of 75% and a specificity of 100% ($p<0.0001$). Choosing the fraction of negative αeff as a criterion, the threshold 0.3 also had a sensitivity of 75% and a specificity of 100% ($p<0.0001$). The good prognostic potential was maintained for patients receiving concurrent chemoradiotherapy. For lymph node GTV, the correlation had low statistical significance. A cross-validation analysis confirmed the potential of the method. In conclusion, the evaluation of the early response in NSCLC patients showed that it is feasible to determine a threshold value for the effective radiosensitivity corresponding to good response. It also showed that a threshold value for the fraction of negative αeff could also be correlated with poor response. These results would therefore allow the early assessment of treatment responsiveness, only one week after the start of the treatment, which would subsequently allow identifying the patients that would benefit from treatment adaptation.

This approach based on repeated examinations performed for the same patient would also provide another advantage. Thus, by using each patient as its own reference no specific assumptions regarding the radiosensitivity of the patient would be required. This is based on the assumption that variation in signal intensities in individual voxels in the same patent would reflect changes in the density of functional clonogenic cells due to cell kill and/or proliferation.

The time point at which the two PET images are taken is one key aspect in this approach. Thus, the optimal results are expected for the case when the assessment of the response is performed no later than two weeks from the start of the treatment when the treatment-triggered inflammatory response in repeated FDG images is not dominating. It addition, an early time point for the assessment of responsiveness would also minimise the impact of the morphological changes associated to tumour shrinkage or progression. These conclusions where derived from a subsequent study to the initial one on NSCLC. Thus, a new cohort of patients for whom the second scan was taken during the third week of treatment was analysed. The optimal window for response assessment was assessed by investigating the ability of the method based on the effective radiosensitivity to predict treatment outcome through a comparison of the results of a ROC analysis for the new cohort of patients, imaged at three weeks, with the results of the previous study in which patients were imaged at two weeks. The results of an ROC analysis on the new cohort of patients showed lack of correlation between either average αeff ($AUC=0.5$, $p=0.7$) or the fraction of negative αeff values ($AUC=0.5$, $p=0.8$) and the overall survival at 2 years for the scan at 3 weeks. This contrasts with the case when the second image was taken during the second week of treatment ($AUC=0.9$, $p<0.0001$). The optimal window for assessing the responsiveness to treatment based on αeff calculations derived from repeated FDG PET scans in NSCLC patients appears to be the second week of the treatment but validation on a larger cohort of patients is warranted.

Based on encouraging results on NSCLC patients, the method for early assessment of tumour response and classification of the patients in good or poor responders based on the effective radiosensitivity calculated from two successive FDG-PET images was tested for feasibility on a sub-group of the H&N patients included in the dose escalation clinical trial in WP8. The results showed that it is feasible to perform the analysis on a different type of tumour with respect to histology and location. A preliminary stratification was performed but it has to be further validated based on all the patients accrued in the H&N clinical trial in WP8. The collection of data from the centres involved in WP8 is therefore still ongoing.

The general methodology for determining the effective radiosensitivity for the patients in this WP is described as follows. The first two successive FDG-PET images will be registered together with the accumulated dose distribution by the time of the second FDG-PET image using a deformable registration algorithm. The effective radiosensitivity, αeff, the operational term that takes into account the delivered dose and the relative uptake of FDG in each voxel giving an indication of the relative density of clonogenic cells, is calculated in each voxel within the gross tumour volume (GTV). Depending on the values of the effective radiosensitivity, the patients will be divided into good and poor responders with respect to the local control and the overall survival at 2 years after treatment. For the patients expected to be poor responders, the distribution of the effective radiosensitivity will be displayed as a map of response overlapping onto the GTVprim and thus allowing for the delineation of the sub-volumes expressing lack of response, hence the sub-volumes that should receive a dose boost as adaptive treatment based on functional imaging.

An example of a map of effective radiosensitivity exported from one slice of the GTV for an H&N target is shown on Figure 10 together with the area that might benefit from a dose boost (in blue).

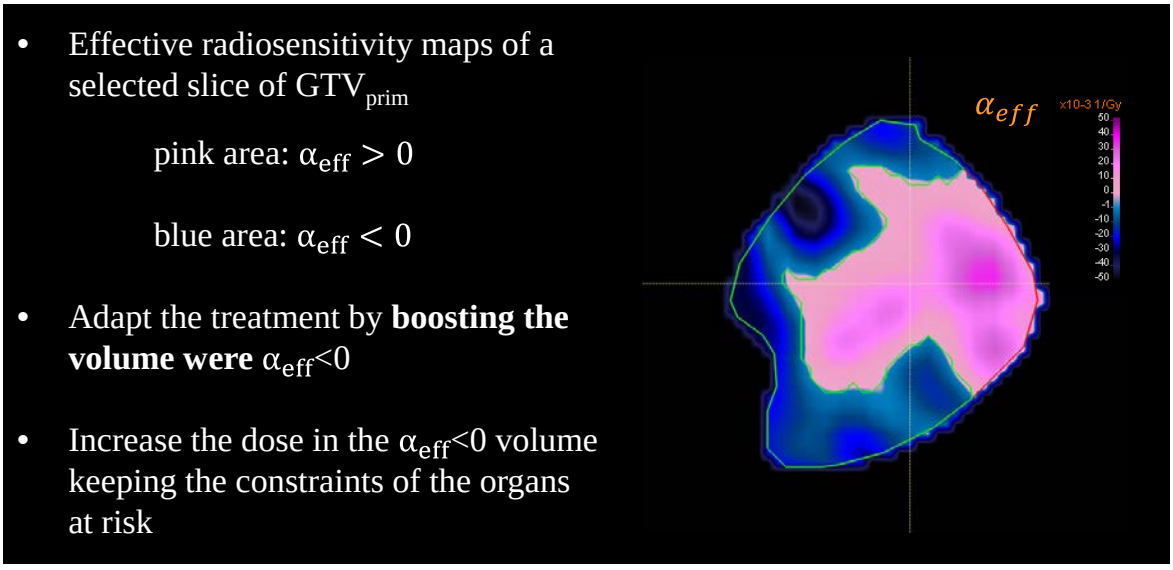

Figure 10. Illustration of the method planned to be used for determining the area that might benefit from a dose boost derived from the calculated map of effective radiosensitivity; the area showing negative values of αeff is identified and delineated as the boost region.

The adaptation of the treatment, however, is not a trivial task as it would require an a priori decision regarding the dose for boosting. Two main options are available for deciding the dose boost: empirical

dose escalation or calculation of the dose that should overcome the lack of responsiveness described by the negative effective radiosensitivity. Some examples of adapted treatment plans involving empirical dose escalation to the sub-volumes expected to lead to poor treatment outcome for the case illustrated in Figure 10 are shown in Figure 11 together with the original photon plan. Two adapted plans are shown, one with photons and one with protons. The empirical dose escalation does not necessarily ensure that the delivered dose boost would overcome the lack of responsiveness. It becomes therefore necessary to use the quantitative information from the effective radiosensitivity, αeff distributions to calculate the dose to be delivered as a dose boost. The effective radiosensitivity values calculated based on the two FDG images include information regarding the surviving and but also the doomed cells - the lethally damaged but not yet dead cells at the time of taking the second FDG-PET image. Therefore, in order to determine a practical effective radiosensitivity to be used to determine the dose boost in the adaptive treatment one needs to correct the αeff for the contribution of doomed cells. The working hypothesis for the current project is that the proportion of doomed cells could be determined if a third FDG-PET image is taken during the third week of the treatment. The methodology for quantifying the dose that should be delivered as a dose boost is described as follows. One FDG-PET image is taken before the start of the treatment as baseline. A second FDG-PET image is taken during the second week of the treatment for determining the responsiveness of the tumour to the treatment and for selecting the patients that should benefit from treatment adaptation. A third FDG-PET image is taken during the third week of the treatment to determine the level of the dose boost to be delivered as adaptive strategy in order to overcome the lack of responsiveness for the patients expected to show local recurrence in absence of treatment adaptation.

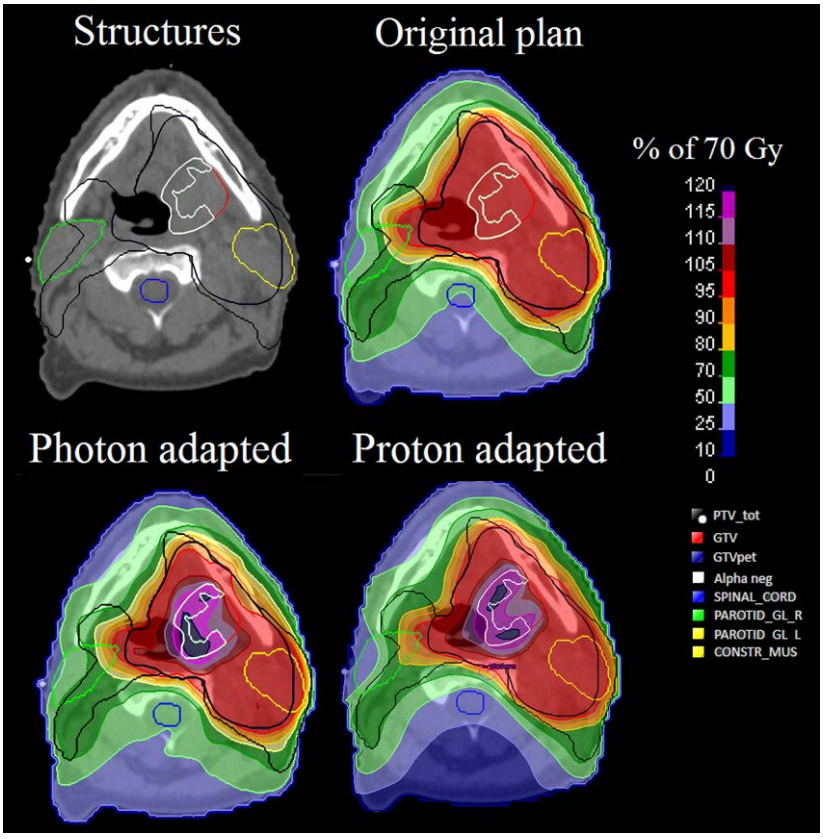

Figure 11. The adapted plans with photons and with protons for a poorly responding H&N cancer patient together with the original photon plan and the delineated structures. In the adapted plans the dose to the region showing lack of responsiveness was escalated. The proposed method therefore has potential to identify candidates for adaptive therapy to increase the rate of local control and also avoid exposing to unnecessary aggressive therapies the majority of patients responding to the standard treatment.

The aim of this work package is to develop and implement three dimensional (3-D) in-vivo dosimetry as an integrated quality assurance (QA) procedure to accurately verify dose delivery applicable to all the major teletherapy systems in the EU.

Every modern treatment machine has an integrated Electronic Portal Imaging Device (EPID) that captures the transmitted radiation that can be used for dosimetric verification of the actual treatment delivery. The objective of this work package was to develop and implement three dimensional (3-D) in-vivo dosimetry as an integrated quality assurance (QA) procedure to accurately verify dose delivery for the clinical trials of work package 7 (PET boost trial) and work package 8 (H&N trial). Furthermore, this forms the basis for a routine implementation of QA procedures of radiation treatments.
There are currently three main vendors of linear accelerators active for radiotherapy (Elekta, Varian, and Siemens). A questionnaire was send out to make an inventory of the specific equipment (software and hardware) used in the consortium for the clinical trials. For the Elekta company, the NKI-AVL (Amsterdam, NL) has developed an in-house suitable QA platform for these treatment machines and implemented this platform at AVL prior to the start of the clinical trials in the ARTFORCE program. The NKI-AVL distributed their platform to be used at the other ARTFORCE partners that use Elekta linear accelerators (Christie Hospital, Manchester, UK). MAASTRO clinic (Maastricht, NL) implemented a 3D dosimetry QA platform for the Siemens treatment machines at the start of the project. The QA platform developed at MAASTRO was upgraded at the beginning of 2012 to also perform 3D dosimetry for the other major teletherapy system (Varian) with their latest type (Varian TrueBeam linear accelerators). A 3D dosimetry QA platform for the Elekta treatment machines was developed and implemented by the NKI-AVL.

For the commissioning of the QA platform software at the other ARTFORCE partners, each centre had to perform dose and EPID image measurements to characterize their specific treatment beam and imaging device which is described in this procedure. These measurements were provided by the centres in the consortium themselves. The QA platform was updated to also incorporate a different type of Varian linear accelerator: Varian Clinac, used at ARTFORCE partners: Villejuif (France), Karolinska (Sweden) and Vall D'Hebron (Spain). The results of the calibration measurements were sent to MAASTRO who performed a fitting procedure to fully characterize the model data. This model data was then be sent back to the participating centre together with the QA software platform.

Furthermore, for in-vivo dose verification of the patient anatomy at the time of treatment calibration of the on-board cone-beam CT imager currently installed on linear accelerators was performed. This step is necessary for accurate dose verification measurements. This verification can be performed using the software developed in this work package (see also Deliverable 4.2 & 4.4) but users/clinics may also use the CBCT calibration procedure for a quick recalculation of the dose distribution inside the new anatomy derived from the CBCT in their own treatment planning system (Figure 12).

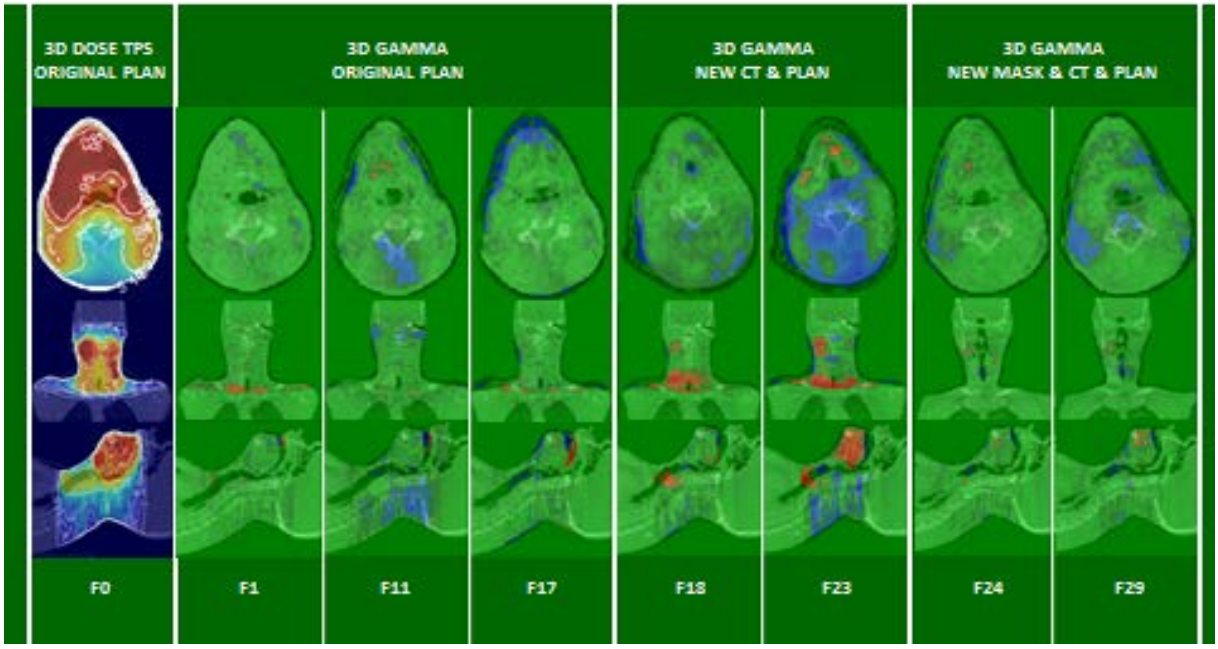

Figure 12 Example of a dose verification procedure Patient in H&N ARTFORCE trial

The calibration procedure and validation of this method has been done (D4.3). Furthermore, the software for the EPID QA was finalized and consists now of two workflows:

1) workflow where the actual delivered dose distribution is recalculated inside the planning CT scan of a patient if no major anatomical differences are expected, and
2) a dose reconstruction inside the online cone-beam CT images based on the delivered radiation fields. An example showing the added value and feasibility to detect even minor dose deviations is shown for a clinical example of the ARTFORCE Head & Neck trial.

An engineer/programmer from MAASTRO went on-site at the ARTFORCE partners to install the QA platform. In all departments, a fully functioning software package was delivered during the site-visit that the clinical team can use directly themselves for verification and QA of the patients included in the ARTFORCE trials.

WP5: Biological markers to predict the response of Head & Neck tumours to Cetuximab or Cisplatinum + RT

Combination of RT with agents targeting the epidermal growth factor receptor (EGFR) is one alternative for patients who are ineligible for the combination Cisplatinum and radiotherapy (CRT) because of older age or co-morbidities. The phase III randomized trial reported by Bonner et al. showed a benefit in in loco-regional control and survival in favour of concurrent bio-radiotherapy (BRT) with Cetuximab compared to RT alone. In a reanalysis of the trial, the benefit of survival outcomes with BRT vs. RT persisted regardless of p16 or HPV status. However, published results of phase III randomized trials which directly comparing cisplatin-based CRT and Cetuximab-based BRT are still awaited, and a fortiori in patients of locally advanced Head & Neck tumours (LAHNSCC) with known human papilloma virus (HPV) status. Therefore, data of 265 patients with LAHNSCC treated with CRT (cisplatin, 100 mg/m2 every 3 weeks, n = 194) or BRT (weekly Cetuximab, n = 71), including 119 patients with known HPV/p16 status were analysed. The combined modality treatment with radiotherapy and Cetuximab for Head & Neck tumours appeared to be less effective than the combination of radiotherapy and Cisplatinum even for patients with patients with known HPV/p16

status (Figure 13). Therefor treatment-specific tumour response predictors were developed for patient selection, i.e. prognostic and predictive biomarkers for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during the treatment.

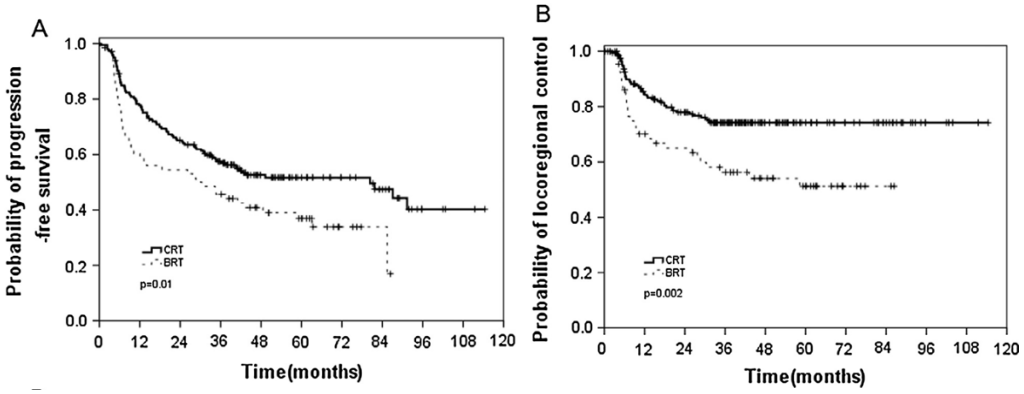

Fig. 13. (A) Progression-free survival and (B) loco-regional control in entire population according to the concurrent regimen of chemotherapy (n = 265). (Ou D1, et a; Oral Oncol. 2016)

Prognostic and Predictive Biomarkers

Prognostic impact of the immune infiltrate. We analysed gene expression pattern indicative of the presence of distinct leukocyte subtypes within four cancer types (breast cancer, colorectal carcinoma, melanoma, and non-small cell lung cancer) and 20 different microarray datasets corresponding to a total of 3471 patients. Multiple metagenes reflecting the presence of such immune cell subtypes were highly reproducible across distinct cohorts. Nonetheless, there were sizable differences in the correlation patterns among such immune-relevant metagenes across distinct malignancies. The reproducibility of the correlations among immune-relevant metagenes was highest in breast cancer (followed by colorectal cancer, non-small cell lung cancer and melanoma), reflecting the fact that mammary carcinoma has an intrinsically better prognosis than the three other malignancies. Altogether, this meta-analysis revealed novel organ-specific features of the immune infiltrate in distinct cancer types, as well as a strategy for defining new prognostic biomarkers.

Prognostic impact of calreticulin expression. A high density of tumour-infiltrating mature dendritic cells (DC) and CD8+ T cells correlates with a positive prognosis in a majority of human cancers. The recruitment of activated lymphocytes to the tumour microenvironment, primed to recognize tumour-associated antigens, can occur in response to immunogenic cell death (ICD) of tumour cells. ICD is characterized by the preapoptotic translocation of calreticulin (CRT) from the endoplasmic reticulum (ER) to the cell surface as a result of an ER stress response accompanied by the phosphorylation of eukaryotic initiation factor 2α (eIF2α). We conducted a retrospective study on two independent cohorts of patients with non-small cell lung cancer (NSCLC) to investigate the prognostic potential of CRT. We report that the level of CRT expression on tumour cells, which correlated with eIF2α phosphorylation, positively influenced the clinical outcome of NSCLC. High CRT expression on tumour cells was associated with a higher density of infiltrating mature DC and effector memory T-cell subsets, suggesting that CRT triggers the activation of adaptive immune responses in the tumour microenvironment (Figure 14). Accordingly, patients with elevated CRT expression and dense intratumoural infiltration by DC or CD8+ T lymphocytes had the best prognosis (Figure 15). We conclude that CRT expression constitutes a new powerful prognostic biomarker that reflects enhanced local antitumour immune responses in the lung. In addition, we analysed the impact of CALR

expression levels detected by microarray finding a positive correlation between CALR and the expression of a metagene indicating the presence of cytotoxic T lymphocytes (CTL) in NSCLC and ovarian cancer.

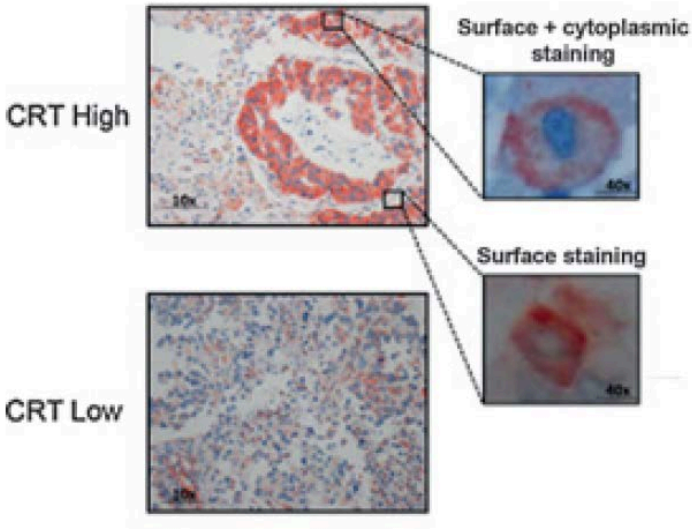

Figure 14 Representative stainings of NSCLC with calreticulin. Calreticulin (CRT)-positive cells are shown in red.

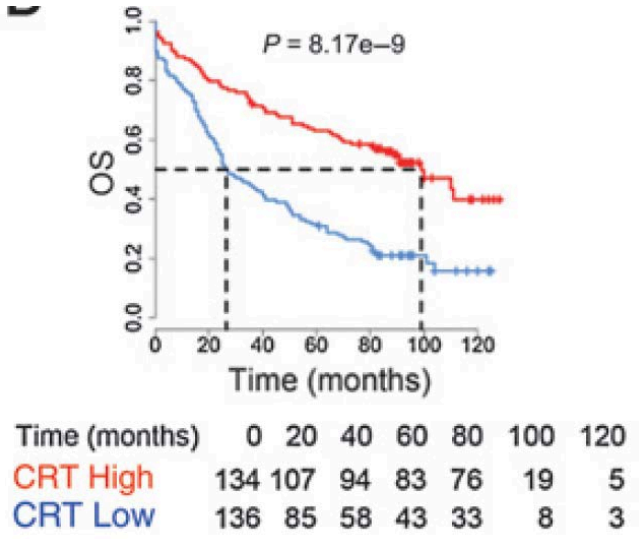

Figure 15A. Derivation cohort. Kaplan–Meier curves of overall survival (OS), upon stratification of NSCLC patients according to median calreticulin (CRT) expression level.

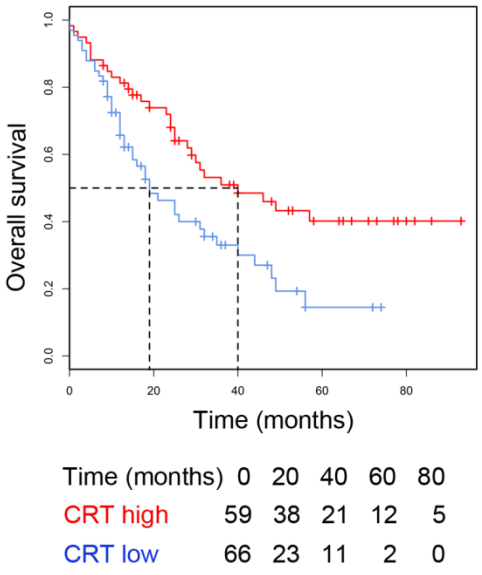

Figure 15B. Validation cohort. Kaplan–Meier curves of overall survival (OS), upon stratification of patients according to median calreticulin (CRT) expression level in patients with NSCLC.

Prognostic and predictive impact of FPR1 mutation. Antitumour immunity driven by intratumoural dendritic cells contributes to the efficacy of anthracycline-based chemotherapy in cancer. We identified a loss-of-function allele of the gene coding for formyl peptide receptor 1 (FPR1) that was associated with poor metastasis-free and overall survival in breast and colorectal cancer patients receiving

adjuvant chemotherapy (but not in non-small cell lung cancer). The therapeutic effects of chemotherapy were abrogated in tumour-bearing Fpr1(-/-) mice due to impaired antitumour immunity. Fpr1-deficient dendritic cells failed to approach dying cancer cells and, as a result, could not elicit antitumour T cell immunity. Experiments performed in a microfluidic device confirmed that FPR1 and its ligand, annexin-1, promoted stable interactions between dying cancer cells and human or murine leukocytes. Altogether, these results highlight the importance of FPR1 in chemotherapy-induced anticancer immune responses.

Prognostic impact of PARP and PDXK expression. Cisplatin-resistant non-small cell lung cancer (NSCLC) cells are often characterized by alterations in vitamin B-related metabolic processes, including the overexpression and hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1) and the downregulation of pyridoxal kinase (PDXK), correlating with elevated apoptosis resistance, as we have reported in the past (Galluzzi et al. Cell Rep. 2012 Aug 30;2(2):257-69; Michels et al. Cancer Res. 2013 Apr 1;73(7):2271-80). Low PDXK expression is an established negative prognostic factor in NSCLC (Galluzzi et al. Cell Rep. 2012 Aug 30;2(2):257-69). We determined by immunohistochemistry the expression of PARP1 and the level of its product, poly(ADP-ribose) (PAR), in two independent cohorts of patients with resected NSCLC. Intratumoural high levels (above median) of PAR (but not PARP1 protein levels) had a negative prognostic impact in both the training (92 stage I subjects) and validation (133 stage I and II subjects) cohorts, as determined by univariate and multivariate analyses. The simultaneous assessment of PAR and PDXK protein levels improved risk stratification. In conclusion, NSCLC patients with high intratumoural PARP1 activity (i.e. elevated PAR levels above median) and low PDXK expression (below median) had a dismal prognosis, while patients with low PARP1 activity and high PDXK expression had a favourable outcome (Figure 15). Altogether, these results underscore the clinical potential and possible therapeutic relevance of these biomarkers. Here, we reproduce the results from derivation and validation cohorts in which PAR and PDXK have been shown to be predictive biomarkers in non-small cell lung cancer:

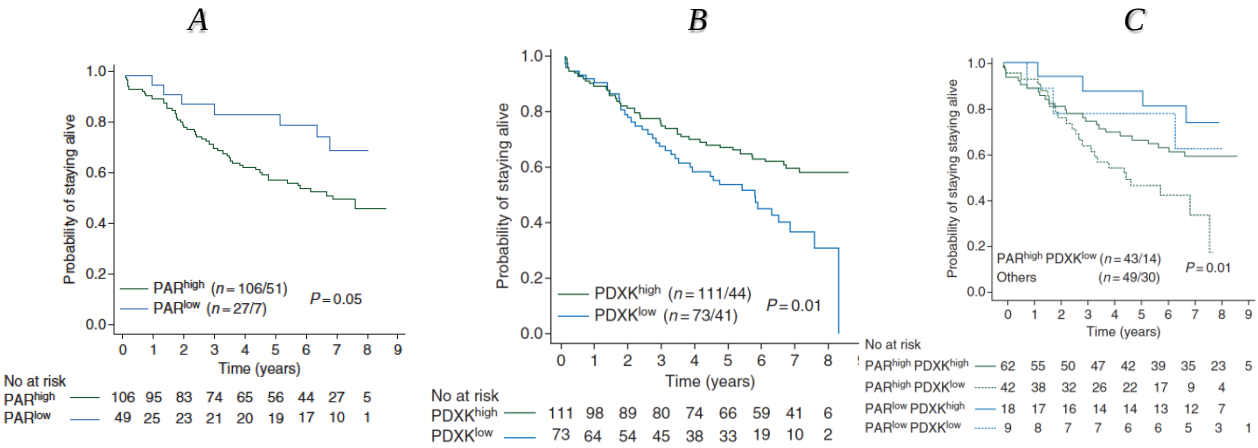

Figure 16 *A*; Validation cohort. Kaplan–Meier curves of overall survival (OS), upon stratification of stage I and II resected NSCLC patients according to median PAR. *B*; Validation cohort. Kaplan–Meier curves of overall survival (OS), upon stratification of stage I and II resected NSCLC patients according to median PAR and PDXK. *C*; Validation cohort. Kaplan–Meier curves of overall survival (OS), upon stratification of stage I and II resected NSCLC patients according to median PAR and PDXK expression depicting four subgroups (PDXKlowPARhigh, PDXKhighPARhigh, PDXKlowPARlow and PDXKhighPARlow). A total of 131 patients' samples were evaluable for PAR and PDXK levels. The subgroup PDXKlowPARhigh was compared with others (PDXKhighPARhigh, PDXKlowPARlow and PDXKhighPARlow). P values were determined by means of the log-rank test.

Prognostic and Predictive value of Radiomics. To investigate prognostic and predictive value of radiomics, three hypoxia-related biomarkers(tumour necrosis, CA-IX and the microvascular density measured as the density of CD34+ vascular structures), tumour infiltrating lymphocytes (CD8+ and FoxP3+), PD-L1 expression, and tumour associated macrophages (TAM) and HLA class I expression in patients with Head & Neck squamous cell carcinoma (HNSCC), we performed imaging analysis and immunohistochemistry of all these biomarkers in 120 patients treated with radiotherapy combined with Cisplatinum(CRT) or Cetuximab (BRT). This study identified potential markers for improving the prognostic accuracy in addition to HPV status and allowing further stratifying patients according to their risk of relapse, could be of interest for patients tailored approaches.

Unsupervised clustering revealed 4 clusters of patients with similar radiomics expression patterns (Figure 17). We established a radiomics signature showing prognosis capacity for predicting 5-year survival in the whole population with an AUC of 0.67 (95%CI, 0.58–0.76). MVA adjusted for clinicopathological factors showed that both the radiomics signature score and p16 significantly predicted for OS and PFS. The combination of radiomics signature and p16 status demonstrated the highest AUC estimate (0.78, 95% CI 0.68-0.88) among all the factors and combinations. when patients were further stratified by radiomics signature score, patients with high signature score significantly benefited more from CRT (vs. BRT) in terms of OS (P = 0.004) and PFS (P = 0.001), while no benefit difference between CRT and BRT in patients with low signature score (P = 0.99, P = 0.90, respectively) (Figure 18).

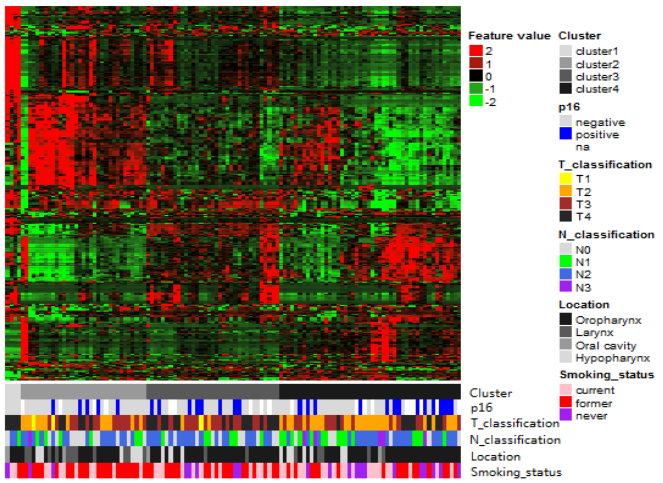

Figure 17 Heatmap displaying Z-scores of the feature values for the 544 radiomics features for the whole cohort (N = 120). Unsupervised clustering revealed 4 clusters of patients with similar radiomics expression patterns. We compared the 4 clusters of patients with clinical parameters, and found significant correlation with T classification ($P = 3.3 * 10^{-5}$), primary tumour location (P = 0.01) and a nonsignificant correlation with smoking status (P = 0.09), whereas no correlation was observed with p16 status (P = 0.19) and N classification (P = 0.22).

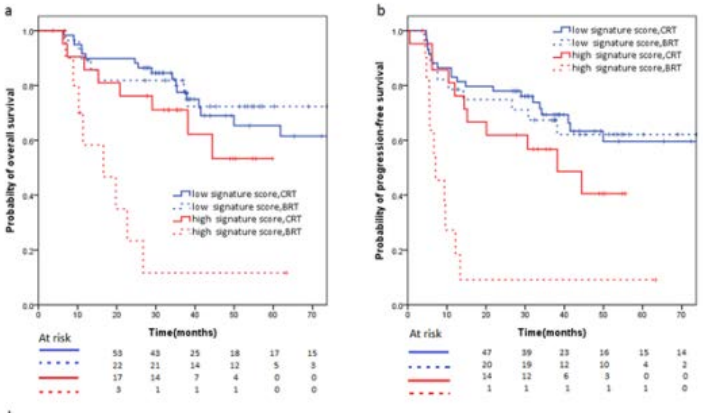

Figure 18. Kaplan-Meier curves for patients stratified by radiomics signature score and concurrent treatment regimen. (a) Overall survival: low signature score, CRT vs. low signature score, BRT, P1 = 0.99; high signature score, CRT vs. high signature score, BRT, P2 = 0.004; (b) Progression-free survival: low signature score, CRT vs. Low signature score, BRT, P3 = 0.90; high signature score, CRT vs. high signature score, BRT, P4 = 0.001.

Microvascular density and CA-IX expression. In tumour biopsy material, we first evaluated the extent of tumour necrosis, the expression level of CA-IX and the MVD measured as the density of CD34+ vascular structures (Figure 19). The correlations between biomarker expressions and clinicopathological characteristics and treatment outcomes were analysed. Multivariate analysis showed that low MVD combined with high CA IX-expression was a significant independent prognostic factor for worse loco-regional control (HR=2.6, 95%CI 1.1-5.0, p = 0.02) in the whole population but not in the p16+ subgroup. Patients treated with CRT had a better LRC than those with BRT independent of MVD or CA-IX expression (Figure 20). The combination of MVD and CA-IX expression might give additional prognostic information in HNSCC patients with known HPV status.

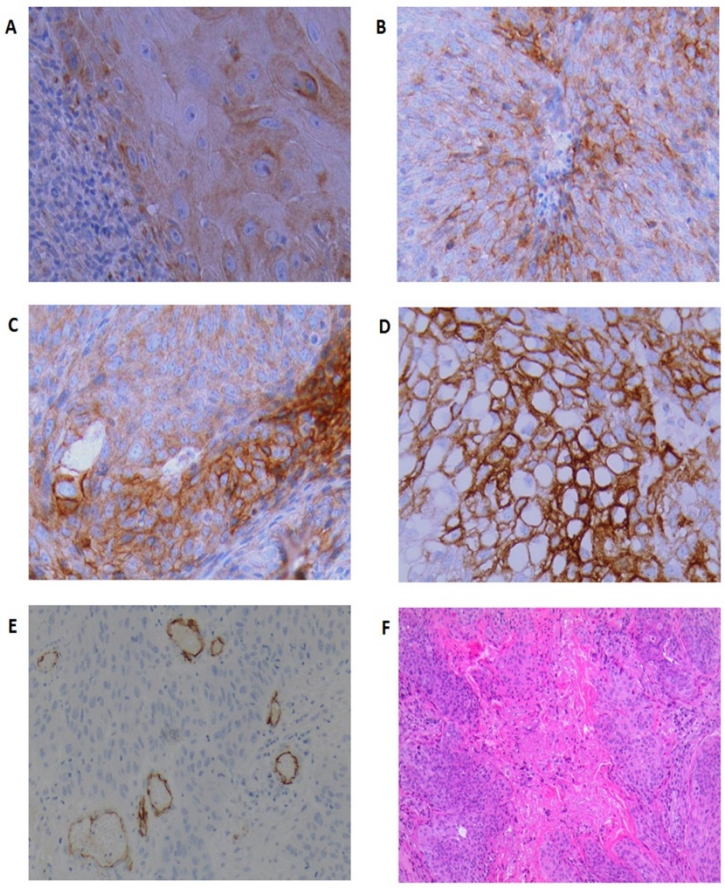

Figure 19. Immunohistochemical images of CA IX, CD34 and necrosis expression. (A): CA-IX negative, no positive cells (400X); (B): CA-IX 1+, rare positive tumour cells(400X); (C) CA-IX 2+, positive clusters consisting of few tumour cells, adjacent to necrotic areas(400X); (D) CA-IX 3+, membrane staining of tumour cells diffuse and widespread, more intense in areas of necrosis (400X); (E) Immunohistochemical image of seven CD34 positive microvessels(400X); (F) Haematoxylin and eosin stained image of abundant tumour necrosis (100X).

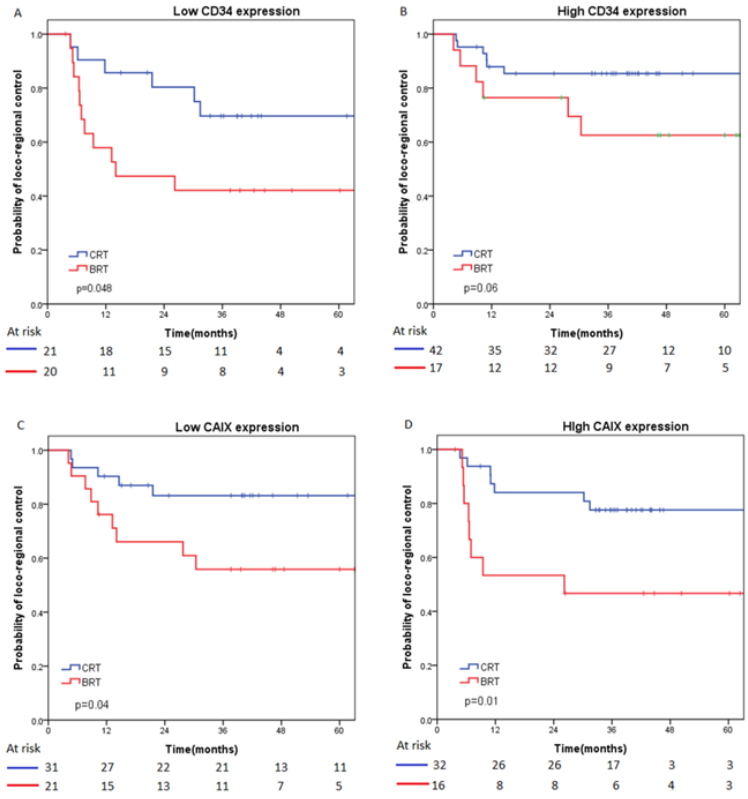

Figure 20. Kaplan-Meier curves of locoregional control in A) low CD34 , B) high CD34 C)low CAIX D) high CAIX expression patients according to the concurrent regimen of chemotherapy.

High CD8+ TIL level as independent prognostic factor. Intraepithelial macrophage expression Immunohistochemistry was also performed for tumour infiltrating lymphocytes (TILs: CD8+ and FoxP3+), and PD-L1 expression to investigate their prognostic values (Figure 21). High CD8+ TILs level was identified in multivariate analysis (MVA) as an independent prognostic factor for improved progression-free survival with a non-significant trend for better overall survival (OS). High FoxP3+ TILs and PD-L1+ correlated with a favourable OS in the uni-variate analysis, respectively, but not in the MVA. In subgroup analysis, CD8+TILs appear to play a pivotal role, p16+/high CD8+TILs patients had superior 5-year OS compared with p16+/low CD8+TILs, p16-/ high CD8+TILs, and p16-/ low CD8+TILs patients. p16+/PD-L1+ patients had improved 3-year OS compared with p16+/PD-L1-, p16-/ PD-L1+, and p16-/ PD-L1- patients (Figure 22). In low CD8+ TILs tumours, 5-year loco-regional control of patients treated with CRT was improved vs. those with BRT (p=0.01) while no significant difference in high CD8+ TILs was observed. The immunobiomarkers may provide information for selecting suitable patients for Cisplatinum or Cetuximab treatment. Additionally, the impact of TILs and PD-L1 of deciphering amongst the p16+ population a very favourable outcome population could be of interest for patients tailored approaches.

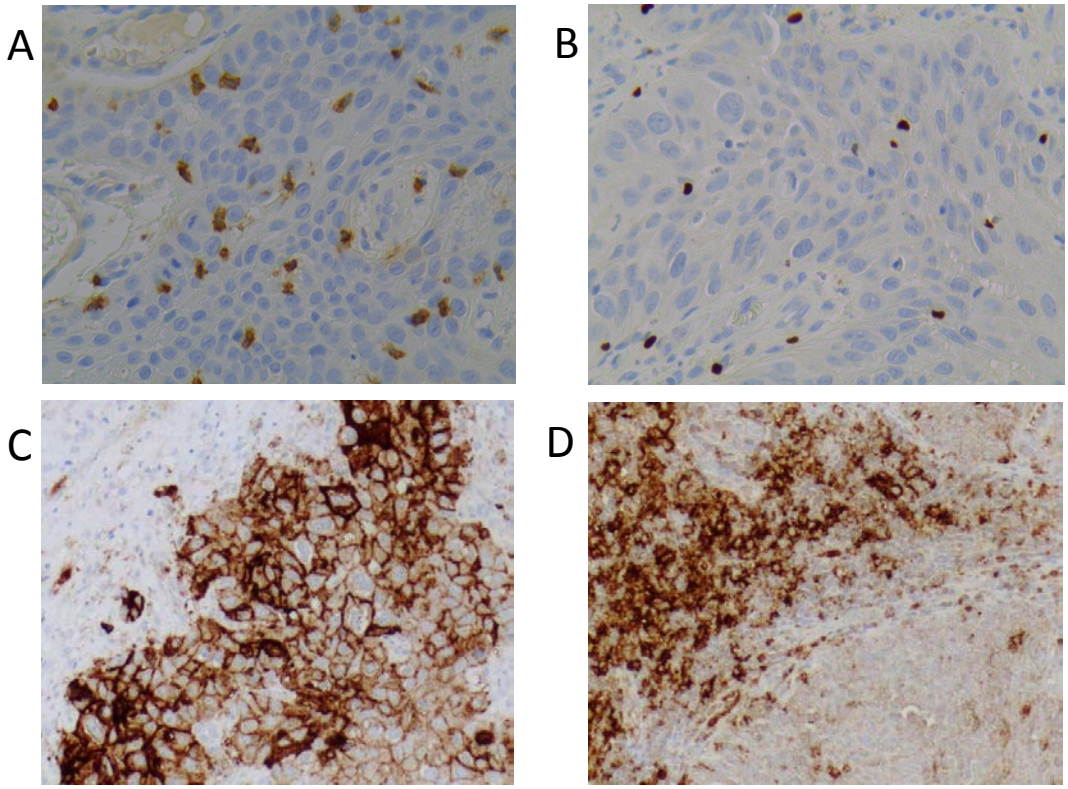

Figure 21. Representative picture of immunohistochemical staining image using A) CD8 and B) FOXP3 specific antibody (400X). Positive cells are stained brown. The exact number of CD8+ and FOXP3+ lymphocytes was evaluated in the tumour site and in the surrounding healthy tissue. C) D) Representative picture of immunohistochemical staining image using PD-L1 specific antibody (200X). Positive cells are stained brown. C) PD-L1 tumour cells positive D) PD-L1 immune cells positive.

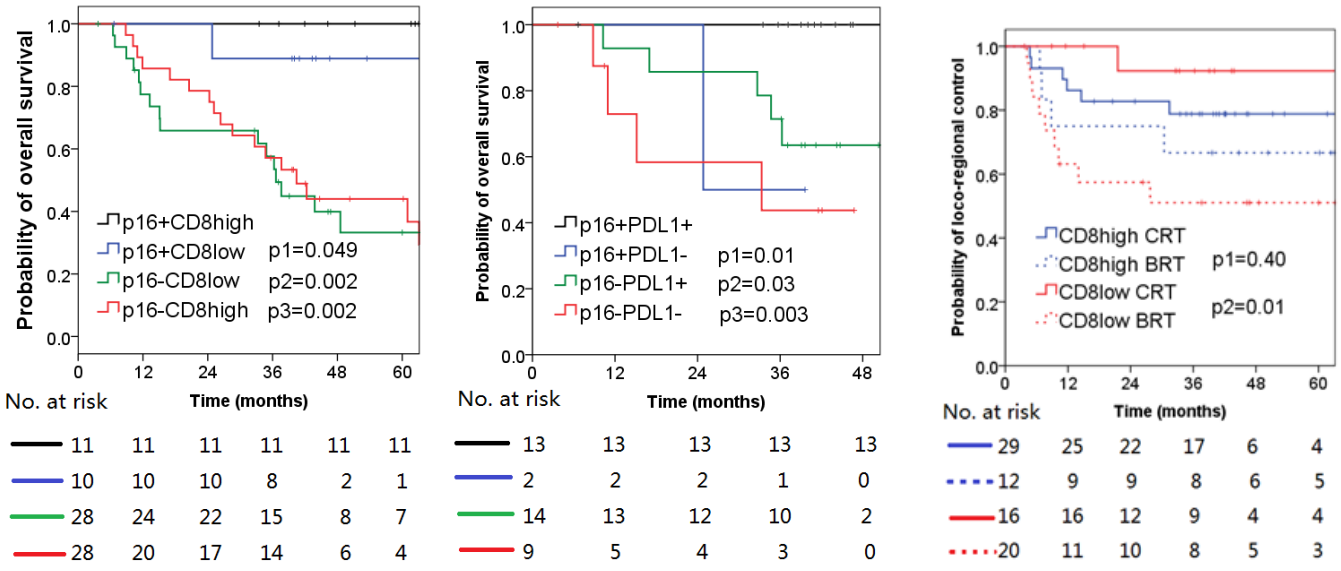

Figure 22. Kaplan-Meier curves of overall survival according to: *A*) CD8+ immune cells infiltration, *B*) PD-L1 expression (1% cut-off level) in HPV/p16 positive and HPV/p16 negative patients and *C*; Kaplan-Meier curves of locoregional control according to CD8+ immune cells infiltration in patients receiving concurrent chemoradiotherapy or bioradiotherapy.

*Biological gene expression markers. S*everal expression signatures have been developed to depict such processes in the past and have been tested for their strength here. RNA sequencing was performed on biopsy material of about 130 advanced HNSCC patients who were treated with radiation combined with cisplatin. Molecular gene expression based biomarkers have been shown to be associated with patient outcome and indeed hypoxia, stem-ness related and others were prognostic in this independent cohort. However novel and independent expression markers are needed to complement and strengthen the prediction. Drug response and DNA repair defect linked expression markers were therefore developed and further improve the detection of poor prognosis patients. These novel markers appear to predict the probability of developing distant metastasis particularly well

WP6 Standardisation and innovative molecular imaging for prediction and decision making
The main objective of the work package was to asses, in a multi-centric setting, innovative imaging modalities allowing better predicting outcome and individualizing. We explored therefor the possibilities of hypoxia imaging and the uptake of immunolabeled EGFR in patients with PET and DCE-CT. Also, radiomics was used as a high-throughput mining of quantitative image features from standard-of‑care medical imaging that enables data to be extracted and applied within clinical-decision support systems to improve diagnostic, prognostic, and predictive accuracy.

Imaging of EGFR with immunoPET: Analysis predictive value of uptake 89Zr-cetuximab
We demonstrated the feasibility of such approach. Due to the limited number of patients, the limited number of events, and the heterogeneity of the received treatments, no conclusions could be drawn regarding the predictive value of 89Zr-cetuximab PET uptake. We believe that this approach will be limited to early clinical research in academic environment due to the complexity of the procedure and the radioprotection issues.
Predictive value of HX4 PET and DCE-CT imaging
A combined analysis of the patients in the PET-boost trial and another trial in non-small cell lung cancer with the same pretreatment imaging protocol (NCT01210378), showed that HX4 PET is a prognostic marker for overall survival. For 58 patients, of which 27 of the PET boost trial, the TBR (SUVmax of the tumour / SUVmean of the aorta) was calculated within the GTV. Patients were divided in hypoxic tumours (TBR > 1.4; n = 37) and non-hypoxic tumours (TBR $\leq$ 1.4; n = 21). A statistically significant survival difference was observed for overall survival (p = 0.0077). For locoregional control, no differences were found between non-hypoxic and hypoxic tumours (p = 0.39) (Figure 23).

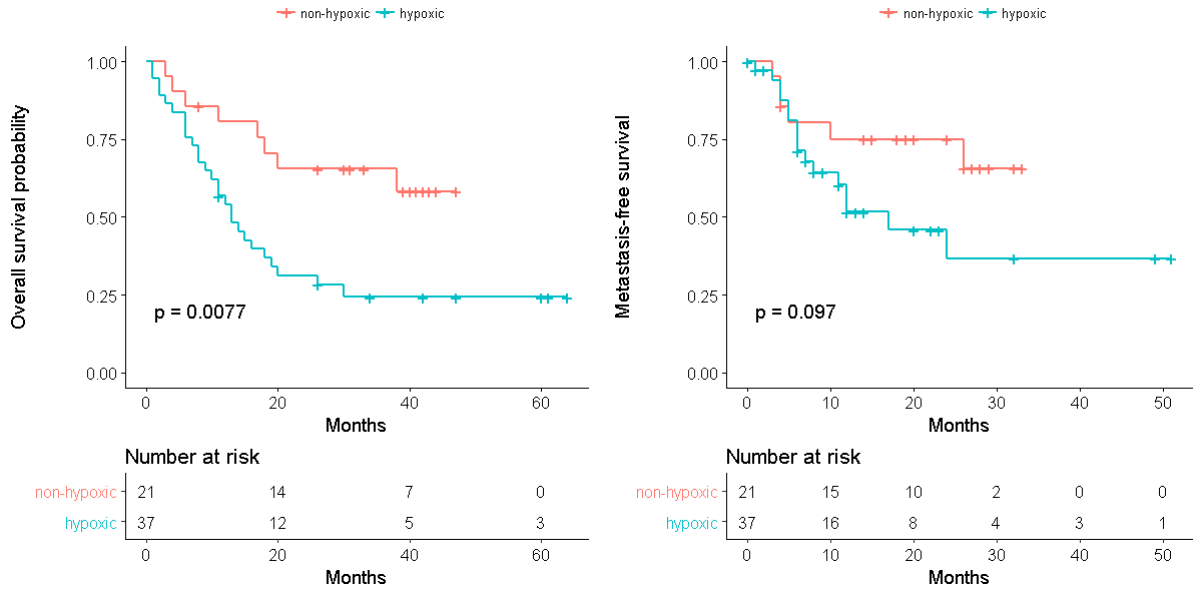

Figure 23: HX4 PET as a hypoxic prognostic marker for overall survival.

We also designed a subregional analysis for multiparametric imaging in NSCLC, and showed the potential of subregion classification as a biomarker for prognosis. This methodology allows for a comprehensive data-driven analysis of multiparametric functional images (figure 23). We then created a data-driven methodology to predict hypoxia levels and hypoxia spatial patterns using CT, FDG PET, and DCE-CT features in NSCLC. The model correctly classifies all tumours, and could therefore, aid tumour hypoxia classification and patient stratification.

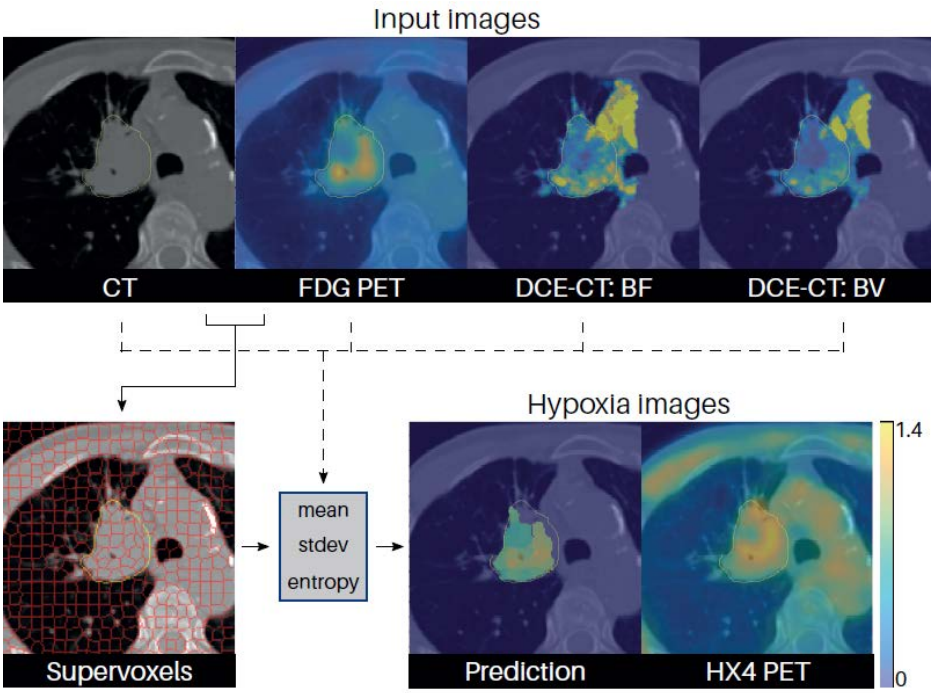

Figure 24 Classification of tumour hypoxia for patient stratification with CT, FDG PET, and DCE-CT in lung tumours.

Radiomics studies on CT and Cone Beam CT: In Head & Neck cancer we developed and externally validated a radiomics signature to predict HPV (p16) status from standard CT imaging (Figure 25).

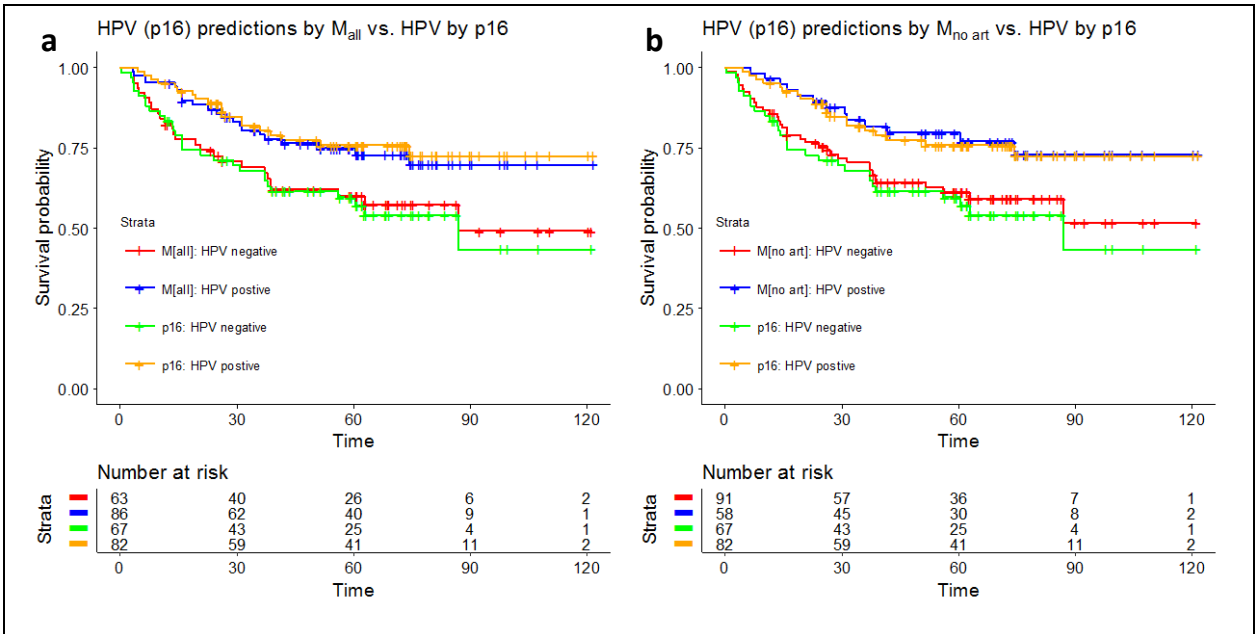

Figure 25 : Kaplan-Meier curves and number of patients at risk for HPV predictions by Mall vs p16 (a) and Mno art vs p16 (b).

This study provides proof of concept that molecular information can be derived from standard medical images and shows potential for radiomics as imaging biomarker of HPV status.

CT radiomics on cone beam CT in Lung cancer: Following our highly cited paper (Aerts et al Nat Commun 2014), in this study we investigated the interchangeability of planning CT and conebeam CT (CBCT) extracted radiomic features. Furthermore, a previously described CT based prognostic radiomic signature for non-small cell lung cancer (NSCLC) patients using CBCT based features was validated. The results show that a subset of radiomic features extracted from CT and CBCT images are interchangeable using simple linear regression. Moreover, a previously developed radiomics signature has prognostic value for overall survival in three CBCT cohorts, showing the potential of CBCT radiomics to be used as prognostic imaging biomarker.

WP7 Dose-escalation by boosting radiation within primary tumour based on a pre-treatment FDG-PET-scan

The clinical PET-boost trial (NCT 01024829): Dose-escalation by boosting radiation dose within the primary tumour on the basis of a pre-treatment FDG-PET-CT scan in stage IB, II and III NSCLC, is an international randomized phase II trial. Patients were accrued in seven hospitals in The Netherlands, Belgium, England, Sweden and Denmark. Inoperable NSCLC patients were randomized to receive a dose of 66 Gy in 24 once-daily fractions of 2.75 Gy with an integrated boost to the primary tumour as a whole (arm A) or to the regions of the 50% SUVmax of the primary tumour on a pre-treatment FDG-PET-scan (arm B) (Figure 26). Patients may have had sequential chemotherapy or received various schemes of concurrent chemotherapy. The primary endpoint of the study was freedom from local failure (FFLF) at 1 year according to the Response Evaluation Criteria of Solid Tumours (RECIST)

version 1.0. Secondary endpoints were acute and late toxicity with respect to fraction dose and volume of normal tissue irradiated, overall survival (OS) and quality of life (QoL). The ultimate aim was to improve the 1-year FFLF from 70% to 85%.

Trial eligibility and work-up

Eligible patients had pathologically proven stage IB-III NSCLC, according to the 2011 TNM-classification. Stage IB and II patients received radiation only and stage III patients sequential chemoradiation (sCRT) or concurrent chemoradiation (cCRT). A SUVmax ≥5 on the pre-treatment FDG-PET-scan was required as well as a minimum primary tumour diameter of 4 cm (to allow boosting of a subvolume). Patients with a loco-regional recurrence in the lung or a second primary tumour at least 3 years following lobectomy were eligible as well.

Between April 2010 and November 2017 a total of 150 patients were registered and 107 randomized, the trial is closed for accrual in October 2017. Forty-three patients were not randomized. In 40 patients dose-escalation to the primary tumour to 72 Gy in 24 fractions was not possible due to exceeding the OAR constraints.

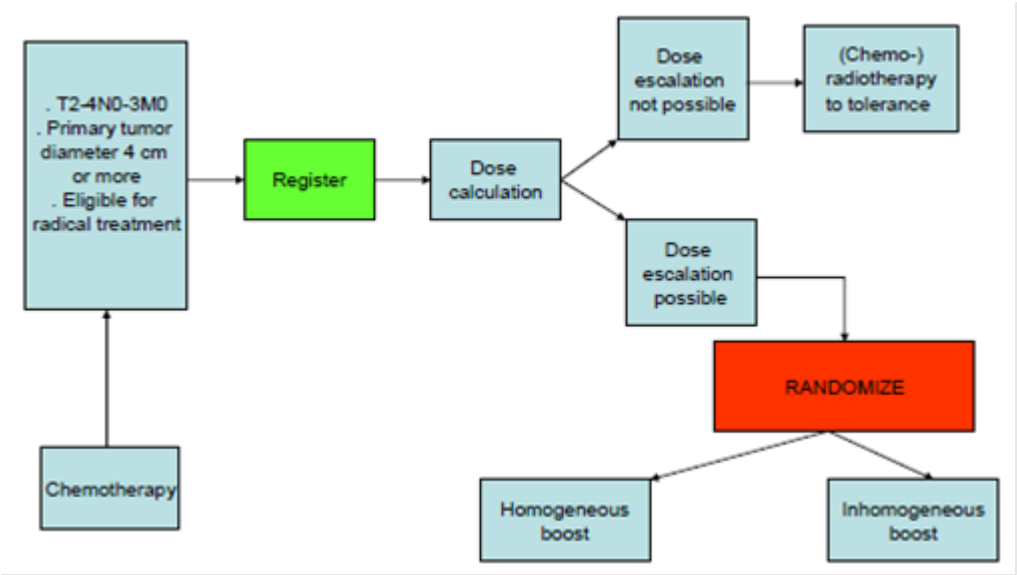

Figure 26 Trial scheme of the clinical PET-boost trial (NCT 01024829): Patients give written informed consent before inclusion. The trial was approved by the medical ethics committees of all participating centres.

Radiotherapy preparation

The delineation of the target volumes and the treatment planning procedure are extensively described in the paper of Van Elmpt et al [1] .

The OAR are delineated according to a predefined protocol using an especially designed Amsterdam-Maastricht normal tissue atlas. Dose constraints are shown in Table 1.

Table 1 – Dose constraints

OAR	**Dose constraint (EQD2)**
Mean lung dose	<20 Gy

Spinal cord (D0.1%)	Max 53 Gy
Esophagus	V35 < 80%
Brachial plexus (D0.1%)	Max 79 Gy
Heart (D0.1%)	Max 94 Gy
Mediastinal envelope	Max 94 Gy

All doses are normalized total dose (EQD2) with an α/β ratio of 2 Gy for spinal cord and plexus, 3 Gy for lungs, heart and mediastinal envelope and 10 Gy for the oesophagus.

Dose limiting toxicities and tumour response
Patients were evaluated weekly during RT to assess acute toxicity by the CTCAEv3.0 criteria. The QoL-questionnaires were collected in week 1 and 5 of treatment. Follow-up consists of appointments after 1 and 3 weeks and every three months thereafter. Toxicity was scored during each visit. An early stopping rule for gastrointestinal, lung, skin or hematologic toxicity requires any grade 3 or higher toxicity occurring in >20% and with the lower bound >10% of the confidence interval. An incidence of 10-15% of fatal haemorrhage was anticipated (because of the selection of large tumour volumes). All toxicities were centrally reviewed and reported to an independent monitoring committee.
Evaluation of tumour response with a CT-thorax and a FDG-PET-scan defined according to RECIST was done 3 months after the end of radiotherapy.

The toxicity results of 61 randomized patients of the first 96 registered were analysed. Overall treatment time was according to the protocol in the majority of the patients.
The majority of the patients received concurrent chemotherapy. The first toxicity results of the PET-boost trial showed that individualized dose-escalation up to normal tissue constraints was feasible and not related with unexpected acute or late toxicity. Therefore, it was concluded that dose-escalated radiotherapy to the primary tumour or regions with high FDG-uptake within the primary tumour did not reveal an unexpected or excess of acute and late toxicity.

After the completion of the trial, a detailed investigation on treatment outcome including toxicity, including DVH parameters, is foreseen at the end of 2018 to allow sufficient follow up of all patient entered in the trial that time, it will also become clear if radiation dose-redistribution may be beneficial and which treatment arm could be further explored in a phase III clinical trial.
In conclusion, the first toxicity results of the PET-boost trial showed that individualized dose-escalation up to normal tissue constraints is feasible and not related with unexpected acute or late toxicity, final analysis will be performed after all patients had at least one year follow up at the end of 2018. (van Elmpt, W., et al.,Radiother Oncol, 2012.)

WP8 Increasing the therapeutic ratio for Head & Neck cancers by pre-treatment selection and dose redistribution
The phase III ARTFORCE clinical trial: A randomised study with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer after initial delays is now well underway it accrued already 181 patients and will finish in the beginning of 2019.The aim is to improve treatment outcome of patients with advanced tumours by enabling tailored radiation to the most active parts of the tumour. Furthermore, the trial aims to improve the quality of life by

withholding ineffective, toxic treatments and decrease community costs by targeting expensive treatments to those who will benefit. On that account, treatment specific tumour response predictors are being developed for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as well as functional and anatomical imaging predictors early during treatment.

In the first approved version of the protocol, the trial consisted of a 2x2 design with four treatment arms, comparing 1. Cisplatinum versus Cetuximab and 2. Standard radiation dose to a dose-redistribution based on the FDG-PET activity. Furthermore, a pre-treatment Zirconium Cetuximab scan is done to improve future patient selection and an additional PET scan in de second week of treatment to facilitate development of biological adaptive radiotherapy in WP3. Patients with locally advanced (at least T3-T4) tumours of the oropharynx, hypopharynx or oral cavity who are fit for treatment with concurrent chemotherapy can be included (Figure 27).

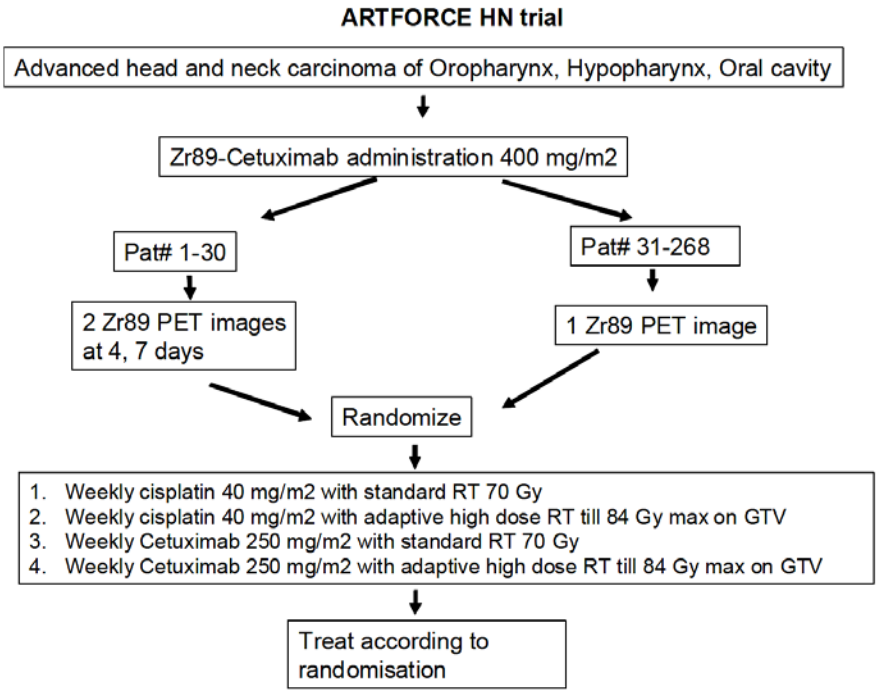

Figure 27: Treatment scheme version V3.0 of the Head & Neck trial.

In 2014, after inclusion of 17 patients, the treatment schedule was amended because Merck stopped the free provision of Cetuximab. The trial was changed to a two arm study comparing standard radiotherapy to dose-redistributed radiotherapy, with in both arms a conventional fractionated radiotherapy scheme with 3-weekly cisplatin. The Zirconium scan was replaced by a radiomics task, as well as an optional hypoxia imaging task with HX4 PET scans (Figure 28).

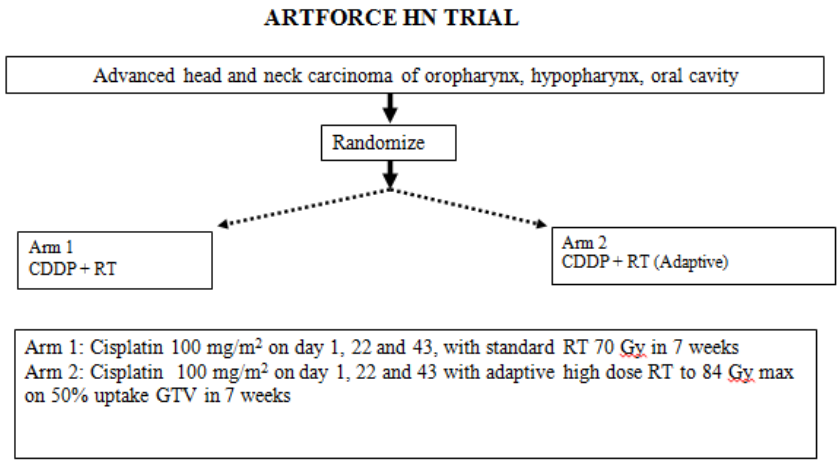

Figure 28: Treatment scheme version 5.0 of the Head & Neck trial

This phase III ARTFORCE clinical trial: A randomised study with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer after initial delays is now well underway it accrued already 181 patients and will finish in the beginning of 2019.There were 3 important, external reasons for this delay. In addition to a number of minor reasons, the first major reason was the termination of the free delivery of Cetuximab due to the expiration of the patent and due to budget limitations within Merck. This was unforeseen and unexpected and had a major impact on the project. The second major reason for delay is the long time it took to obtain Medical Ethical Approval and Regulatory Affairs Approval for the trials in all participating centres in the different countries. The third major reason for delay was slow accrual caused by severe restrictions due to radiation safety imposed on the daily life of the patients treated with radiolabeled Cetuximab. The protocol for this Head & Neck trial has been adapted by removing the imaging studies with the radiolabelled Cetuximab. The adapted protocol does not impose the significant life-style restrictions on the patients (they can now freely interact with their close relatives) and this is improving accrual. To further improve accrual, we have invited more hospitals to join ARTFORCE as a partner (UMC Utrecht, EMC Rotterdam and UMC Groningen). These centres were selected because of their large Head & Neck cancer population. The Head & Neck trial will be finalized in the beginning of 2019.
Participants: Maastro clinics, Maastricht, The Netherlands; University Medical Center Utrecht, Utrecht, The Netherlands; University Medical Center Groningen, Groningen, The Netherlands ;Erasmus Medical Center, Rotterdam, The Netherlands; The Christie NHS Foundation Trust, Manchester, United Kingdom; Institute Catala de la Salut, Vall d'Hebron, Barcelona, Spain; Gustave Roussy Cancer Institute, Villejuif, France; Karolinska Institute, Stockholm, Sweden; Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, The Netherlands

Primary Endpoints are:
Comparison of conventional RT versus PET-GTV adapted RT in terms of loco-regional control at two years. The contrast between both radiotherapy arms will be analysed by means of the log-rank test adjusting for the strata used at randomization. The primary analysis will be based on the intention-to-treat principle. In addition, 'protocol-correct' analysis will be performed on eligible and evaluable patients only, following their actual treatment.

Toxicity at 2 years is also primary interest of this study. Toxicity (worst grade), adverse and serious adverse events will be compared between both arms by tests of proportions. Patients will be evaluable for toxicity evaluation when at least one administration of chemotherapy / radiotherapy has been given.

Secondary Endpoints are:
Quality of life: The EORTC QLQ-C30, the HN35 and the EQ-5D-5L will be scored according to the standard procedures recommended by the EORTC or the EuroQoL Group respectively. The statistical significance of the difference of observed mean changes between treatments in scores over time will be tested by means of the mixed effect modelling procedure (SAS proc mixed). All patients with at least one follow up will be included in the estimated model of change over time. Swallowing function preservation at one year: Swallowing preservation will be measured by PRG dependency at one year and ability to swallow solid food, drinks and aspiration as measured on video fluoroscopy, CTC toxicity scores and quality of life forms at this time point. The contrast between both radiotherapy arms will be analysed by means of the chi-square test. In addition, explorative subgroup analysis will be performed adjusting for the pretreatment swallowing by means of logistic regression analysis.
Progression Free Survival and Overall Survival: The contrast between both radiotherapy arms will be analysed by means of the log-rank test adjusting for the strata used at randomization. The primary analysis will be based on the intention-to-treat principle. In addition, 'protocol-correct' analysis will be performed on eligible and evaluable patients only, following their actual treatment.
This trial is interlinked with the translational research work packages in the ARTFORCE project: In WP3: Imaging (PET) and dose distributions will be provided for analyses planned in WP3 to develop biological adaptive radiotherapy. In WP5: Biopsies will be provided for analyses planned in WP5 for prognostic and predictive parameters for response to cisplatin-RT. In WP6: Imaging (CT, MRI, PET) will be provided for analyses planned in WP6 on standardisation and innovative imaging for prediction and decision making. The value of hypoxia imaging with HX4-PET will be analyses together with Maastro to evaluate whether survival, local tumour progression and pattern of relapse within the tumour correlate with [18F]HX4 uptake.

WP9 Distribution of knowledge and expertise
Main results and highlights
ESTRO achieved its goal in the distribution of knowledge and expertise on the ARTOFORCE project as evidenced by:
-dissemination of results of the project at all ESTRO annual congresses from 2011 to 2017, a total of 7 congresses whose abstracts are published in the society journal with an open access.
-ensuring that results of the project were also released 5 times in the ESTRO newsletter, 2011 to end 2016, with open access to the public but also to ESTRO's 12k contact database.
-acquisition of expertise was guaranteed through the offer of grants to attend courses or transfer of knowledge to young scientist working on the project through exchange visits. In total 7 grants to a course and 5 exchange visits were realised.

Potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

General impact

Cancer is nowadays the most common cause of death in Europe. Radiotherapy plays an important role in the curative and palliative treatment of cancer and is applied in nearly half of the patients. Progress in a better and safer radiotherapy will therefore have a major socio-economic impact considering the large number of European cancer patients. Applying new image guided targeted radiotherapy approaches with higher radiation doses to the tumour, while sparing the surrounding normal tissues, will lead to higher cures rates without increasing the side effects. However advanced new quality assurance programs are required to allow safe introduction into the clinical practise of the newly developed targeted radiotherapy treatments.

Withholding ineffective, toxic treatments and by targeting expensive treatments to those who will benefit will lead to decreased community costs. To prevent that patients suffer from severe side effects without benefiting from a certain treatment regimen: specific and sensitive biomarkers are urgently needed to support treatment decision. Immunological and genetic predictors for Cisplatinum and radiation sensitivity, as well as functional and anatomical imaging predictors with CT-PET-MRI will provide important prognostic information during the treatment. Such treatment-specific tumour response predictors need to be developed and validated for patient before safe introduction in the routine clinical practise selection.

Optimizing Radiotherapy with validation in randomized clinical trials

The ARTFORCE project succeeded by introducing very sophisticated imaging and radiotherapy tools into daily clinical practice to improve treatment outcome of patients with advanced Head & Neck and lung tumours. The work carried out in this project aimed at higher tumour control rates with improved quality of life, by using MRI and PET imaging for targeting on the most radioresistant part of the tumour. This was achieved by enabling tailored irradiation to the most active parts of the tumour with the adapted image intensity modulated guided radiotherapy (IGART) based upon CT, MRI and PET imaging before and during treatment. For precise delivery of the radiation dose novel on line image guided adaptive irradiation techniques were developed and very advanced quality assurance methods with daily CT cone beam imaging and in vivo dosimetry. This allowed safe delivery of higher tumour radiation doses with sparing of the normal tissues. These methods were tested and approved within two major clinical trials:

- A randomised Phase III trial with Cisplatinum or Cetuximab and standard or adaptive high dose radiotherapy for advanced Head & Neck cancer
- Dose-escalation by boosting radiation dose within the primary tumour on the basis of a pre-treatment FDG-PET-CT scan in stage IB, II and III NSCLC: A randomized phase II trial

Withholding ineffective, toxic treatments and to decrease community costs by targeting expensive treatments to those who will benefit was another objective of this project. We showed that the combined modality treatment with radiotherapy and Cetuximab for Head & Neck tumours was less effective than the combination of radiotherapy and Cisplatinum even for patients with patients with known HPV/p16 status. Therefor treatment-specific tumour response predictors were developed and tested for patient selection, i.e. genetic predictors for Cisplatinum and radiation sensitivity as, well as functional and anatomical imaging predictors early during the treatment.

It is anticipated that both clinical trials carried out in work package 7 and 8 will lead to a higher cure rate for patients with, without increasing side effects, leading therefor to a better quality of life. A final prove for this statement is foreseen in 2019, especially as the Head & Neck trials will be completed in that year. At that time, the validation will occur of the immunological, genetic and imaging biomarkers.

Development of Quality Assurance procedures

Precise treatment of tumours is important to achieve optimal results of the radiotherapy. Quality assurance programs are therefore implemented at various levels during the radiotherapy procedures. In this work package an individualized QA program was developed to guarantee the actual delivery of the radiation to the precise location in the human body. As treatment schedules may span over multiple weeks, the anatomy of the patient may change due to weight loss or tumour shrinkage as a result of therapy. On-board imaging capabilities of the cone-beam CT imager can now be used to capture the anatomy at the time of treatment. Furthermore, an on-board imaging device (flat panel electronic portal images, EPID) was calibrated to act as a dosimetry device to capture the actual radiation delivered during the treatment. A QA platform was developed that combined of both this anatomical information and treatment (dosimetry) to make a comprehensive verification platform that works for all major linear accelerator vendors. The developed platforms in this project proofs its potential and need of these tools for wide scale and multi-centric implementation of quality assurance in radiotherapy procedures. This is expected to be beneficial at the individual patient level to detect treatment errors and allows for corrective procedures to mitigate potential risks. These patient individual QA techniques will allow to early detect potential over dosage of critical organs at risk that (if uncorrected) will lead to (major) subsequent toxicities that increase the burden on the health care system and detriments the quality of life of patients.

Second impact will be on the cohort level of all cancer patients treated. The developed techniques will allow to improve the quality of the entire treatment procedure by assessing generic improvement in class solutions and treatment techniques driven by information of the actual treatment. A next step will be taken to better estimate the actual delivered dose to tumours to reduce the uncertainty in dose-response relationships. This uncertainty reduction allows to optimize the dose prescription (how much dose is needed to eradicate a tumour) and dose delivery (which techniques are most robust to reach the prescribed dose) for large groups of cancer patients including. Furthermore, the developed techniques are directly generalizable to also allow verification of other indications that the investigated head-and-neck and lung cohort in the ARTFORCE trials.

Adaptations during Radiotherapy

In addition to the clinical potential to improve the outcome of Head & Neck or lung cancer patients by early assessing their responsiveness to the treatment and adapting the treatment accordingly, the work performed in WP2 and WP3 might prove to have a higher socio-economic impact and wider societal implications as highlighted below:

- Optimising the efficiency of treatment by selecting patients in need of adaptive radiation therapy
- Improving quality of life both in responders to standard treatment by preventing unnecessary dose escalations and in non-responders by referring them to more effective adapted treatments with photons or with protons
- Decreasing hospital and community costs by recommending adaptive, and potentially more expensive, approaches only for those patients who will benefit from them

Treatment selection with the novel biomarkers and imaging tools

A considerable proportion of patients is diagnosed at a late stage with Head & Neck or Lung cancer and is treated with a combination of radiotherapy and Cisplatinum. This combination is effective, although not all patients benefit and less than half of the patients will be cured. In addition, many suffer from severe side effects without possibly benefiting from the treatment. Known prognostic factors are site, tumour volume, HPV status and the extend of hypoxia but are limited in their robustness and accuracy to predict response. Specific and sensitive biomarkers can support new treatment decision aids are therefore urgently needed. Biological gene expression markers can help to predict the response of Head & Neck tumours to radiotherapy combined with Cisplatinum treatment. Molecular gene expression based biomarkers have been shown to be associated with patient outcome and indeed hypoxia, stem-ness related and others were prognostic in this independent cohort. However novel and independent expression markers are needed to complement and strengthen the prediction. Drug response and DNA repair defect linked expression markers were therefore developed and further improve the detection of poor prognosis patients. Further exploration of biomarkers, revealed novel predictors and prognostic biomarkers, including in a multi-centric setting tested innovative imaging modalities with radiomics. Within this project proof of concept was provided that molecular information can be derived from standard medical images with radiomics and it provided prognostic for patients with Head & Neck cancer. Validation will occur of the immunological, genetic and imaging biomarkers after completion of the clinical trials in 2019.

Distribution of knowledge and expertise

ESTRO ensured the dissemination of the ARTFORCE project results and the clinical and social consequences, that arise from the scientific conclusions of the project via its communication channels: its annual congress targeting the scientific community and industry (about 6k attendants and more) and its electronic newsletter available not only to the 12k contacts in its database but the public at large. ESTRO annual congresses are interdisciplinary hence are attended by the oncology community beyond the radiation oncology world. Furthermore, abstracts from the annual congress are published in the Society Journal: Radiation & Oncology, access open to the public. ESTRO conferences attract more than 6000 individual and company delegates in the field of radiation therapy and beyond. Distribution of knowledge and expertise was further guaranteed through the grants established for courses and exchange visits for young scientists to increase their knowledge all to the benefit of the ARTFORCE project. This was accompanied with the publication of newsletters and 222 peer reviewed papers on this ARTFORCE project.