FMISO-based adaptivní radioterapie nádorů hlavy a krku – prospektivní multicentrická studie

Project introduction including relevance and topical issues

Curative treatment of head and neck cancer

Approximately 1 800 patients are diagnosed annually with squamous cell head and neck cancer (HNC) in Czech Republic.

Historically, radiation therapy (RT) alone was the standard nonsurgical therapy for locally advanced disease. Unfortunately, RT regimens result in local control rates of only 50% to 70% and disease-free survivals (DFSs) of 30% to 40%. Most randomized clinical trials show the superiority of combined RT and chemotherapy to RT alone for the treatment of locally advanced, nonmetastatic HNC. A meta-analysis of individual patient data from >17,346 participants in 93 trials conducted from 1965 to 2000 (Meta-Analysis of Chemotherapy in Head and Neck Cancer [MACH-NC]) demonstrated that the use of radiotherapy and concurrent chemotherapy resulted in a 19% reduction in the risk of death and an overall 6.5% improvement in 5-year survival compared to treatment with RT alone. This benefit was predominantly attributable to a 13.5% improvement in locoregional control. (1)

RT and concurrent chemotherapy represent the most commonly used strategy and is a biologically attractive approach because some chemotherapeutic agents may both radiosensitize cells and provide additive cytotoxicity.

Hypoxia

The significance of a lack of oxygen was demonstrated in the first radiobiologically oriented clinical study implicating the importance of environmental parameters in the outcome of radiotherapy by Schwarz in 1909. (2) On the contrary, Müller reported that tissues in which the blood flow was stimulated by diathermia showed a more prominent response to radiation. (3) These observations led Gray in the early 1950s to postulate that oxygen deficiency (hypoxia) is a major source of radiation resistance. (4) Hypoxia has been directly identified in most animal solid tumors, with the values ranging from less than 1% to well more than 50% of the total viable cell population. (5)

Estimating hypoxia in human tumors has generally involved the use of indirect methods. The endpoints included immunohistochemical estimates of intercapillary distance, vascular density, and distance from tumor cells to the nearest blood vessel; oxyhemoglobin saturation determined using cryophotometry or noninvasively with magnetic rezonance imaging (MRI); measurements of tumor perfusion using MRI, computed tomography (CT), or positron emission tomography (PET). (6-14) With the finding that hypoxia could upregulate gene/protein expression, it was suggested that endogenous markers could be used to identify hypoxia. (15-17)

More popular techniques for identifying hypoxia involve measurements of the binding of exogenous markers. This can be achieved following immunohistological analysis of biopsied sections using pimonidazole or EF5 or noninvasively with PET, single-photon emission computed tomography (SPECT), or MRI analysis of radioactively labeled nitroimidazoles (^{18}F labeled misonidazole, ^{123}I labeled azomycin arabinoside). (18-23)

The relationship between pre-treatment measurements of tumor oxygen tension (pO2) and survival in advanced head and neck cancer was proved by Nordsmark in 2005. In this international multicenter study in head and neck cancer patients, their tumor's pO_2 was measured before radiation therapy and was found to correlate with overall survival - the patients with lower tumor oxygenation status did significantly worse. (24)

Another evidence for the existence of hypoxia in human tumors comes from the large number of clinical trials in which hypoxic modification has shown benefit. The drugs reaching clinical evaluation include metronidazole, misonidazole, benznidazole, desmethylmisonidazole, etanidazole, pimonidazole, nimorazole, ornidazole, sanazole, and doranidazole.

The second Danish Head and Neck Cancer study (DAHANCA 2) found a highly significant improvement in the stratification subgroup of pharynx tumors using misonidasole. (25) Whereas the two multicenter trials in head and neck cancer using etanidazole showed no benefit, studies with the nimorazole given to patients with supraglottic and pharynx carcinomas (DAHANCA 5) showed a

highly significant benefit in terms of improved locoregional tumor control and disease-free survival rates, confirming the result of the DAHANCA 2 study. (26, 27)
The recent meta-analysis of randomized clinical studies in squamous cell carcinoma of the head and neck using hypoxic radiosensitizers to improve radiotherapy clearly showed that radiosensitizer modification of tumor hypoxia significantly improved locoregional tumor control and overall survival, with odds ratios of 0.71 and 0.87, respectively. (28)

One of the major factors influencing the delivery of oxygen to tumors is the concentration of hemoglobin. The low hemoglobin concentration in general has a negative impact on tumor radiation response. In a review of 51 studies involving 17,272 patients, the prognostic relationship between hemoglobin concentration and local tumor control was analyzed. Of these, 39 studies (14,482 patients) showed a correlation, whereas only 12 (2790 patients) did not. (29) Unfortunately using transfusion to raise hemoglobin levels in two randomized trials (Canadian trial, DANAHANCA 5) did not improve clinical outcome. (30, 31). The concept of using EPO to correct for anemia has also been tested in several clinical trials. However, although low hemoglobin can be effectively and safely improved by EPO, patients treated with radiation and EPO had a poorer outcome than the control arms not treated with EPO. (32, 33)

Hypoxia evaluation using FMISO
The mechanism of 18F-MISO accumulation has been appropriately described by Padhani et al. (34)
The partition coefficient of ^{18}F-MISO (Fig. 1) nearly equals one so the molecule freely diffuses into all cells. Once ^{18}F-MISO is in an environment with electron transport occurring (viable tissues), the –NO2 substituent (which has a high electron affinity) takes on an electron to form the radical anion reduction product. If O_2 is also present, that electron is rapidly transferred to oxygen and ^{18}F-MISO changes back to its original structure and leaves the cell. However, if a second electron from cellular metabolism reacted with the nitroimidazole to form the 2-electron reduction product (Fig. 2), the molecule reacts non-discriminately with peptides and RNA within the cell and becomes trapped. Thus, the retention of ^{18}F-MISO is inversely related to the intracellular partial pressure of O_2.

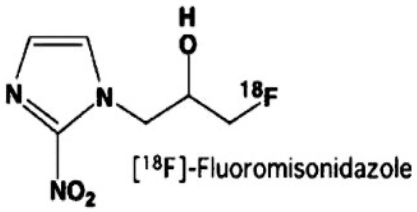


Fig 1 ^{18}F-MISO

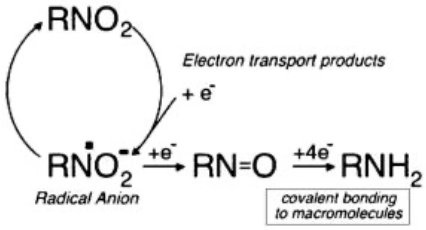


Fig. 2 ^{18}F-MISO covalent binding in hypoxia

Dynamic ^{18}F-MISO PET images were acquired and decay-corrected time-activity curves were generated. Hypoxic tissues showed a continuous increase of radiopharmaceutical accumulation (Fig. 3). On the contrary, the accumulation of radiopharmaceutical was stable in non-hypoxic tissue. (Fig 4). The time-activity curves (TAC) in the figure show that the contrast between hypoxic and surrounding tissue increases in time. However, the quality of the PET images decreases in time after the injection because of a short ^{18}F half-life (110 minutes). For these reasons, the acquisition of a static ^{18}F-MISO PET scan is usually started 150 min p.i.

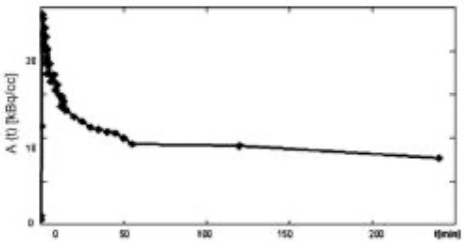


Fig. 4 ^{18}F-MISO TAC in non-hypoxia

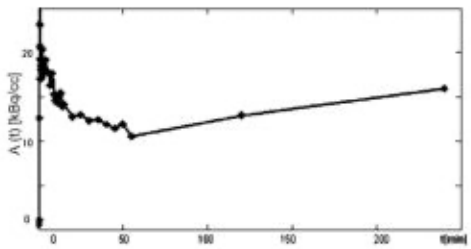


Fig. 4 ^{18}F-MISO TAC in hypoxia

The studies published so far differ partly in the way the hypoxic area is delineated. The target-muscle ratio (TMR) was most often applied to define the boundaries of the hypoxic tumor volume (HTV). A TMR value of 1.4 has been used in recent papers. The metabolic tumor volume (MTV) will be estimated from ^{18}F-FDG PET/CT images.

Locoregional relapse

Loco-regional recurrences after chemoradiotherapy originate from the initial gross tumor volume (GTV) containing hypoxic subvolumes. (35) Nishikawa confirmed, that the uptake of FMISO in the recurrent region is significantly higher than that in the non-recurrent region. (36) Thus, optimization of radiotherapy in hypoxic subvolumes represents one of the most important **unmet clinical need**.

Dose painting by numbers can be the elegant way to individualize radiotherapy by functional imaging such as PET, which could overcome the resistance of hypoxic HNSCC. (37-39)

Hypothesis

Locoregional failure constitutes the predominant recurrence pattern, and most fatalities result from uncontrolled local and/or regional disease. Besides tumor volume correlating with cell number per tumor, hypoxia is a important biological parameter for tumor progression. Hypoxia increases radioresistance and is a predictive factor for local failure, because retrospective data suggest that loco-regional recurrences after chemoradiotherapy originate from the initial GTV containing hypoxic subvolumes.

Hypoxia occurs in about 80% of head and neck tumors. Based on experimental and clinical data, hypoxia is a useful parameter for pretherapeutic stratification. Moreover, hypoxic subvolumes of tumors can be evolving as target volumes for radiotherapy (“dose painting”) in hypoxia imaging-based dose escalation.

The location of the relapse site often corresponds to the foci of tumor hypoxia that may change in location and intensity. These radioresistant regions can be detected with FMISO PET/CT. The escalation of dose to hypoxic tumors may improves outcomes.

These considerations lead to hypothesis that personalized dose escalation by 10-15% to the hypoxic tumors may be safe and may result in improved locoregional control.

Aims

1. **Unification of reconstruction parameters of each PET/CT scanner.**
   (The target reconstruction settings must fulfil the EARL version 1 criteria.)
2. **To establish prospective register for patients with hypoxia treated using dose escalation protocol and for patients without hypoxia treated with conventional protocol**
3. **To analyse patient´s and physician´s reported toxicity, complete response and locoregional control and comparison with cohort treated using standard dose fractionation**

Statistical Considerations:

The patient group with escalated dose will be compared with a retrospective cohort using a standard dose. The complete response (CR) rate comparison would require a sample size of 40 patients with escalated dose (and 80 with standard dose) to achieve a power of 80% and a level of significance of 5% for declaring that the CR rate in the experimental group is not inferior to the control group at -15% non-inferiority margin. With an expected drop-out of about 10%, a total of 45 patients is planned to be included.

Patient characteristics will be described using standard summary statistics, i.e., median and interquartile range or mean and standard deviation for continuous variables and frequencies and proportions for categorical variables. Fisher's exact test will be used for comparison CR and G3 toxicity rates between groups. Depending on the data type, common statistical tests will be used for group comparison. The locoregional control will be evaluated with regard to follow-up using the

Kaplan-Meier estimate of the survival function and compared using the log-rank test. All statistical analyses will be performed employing a common significance level of 0.05.

Justification of aims and novelty of the project

In accordance with the announced program of the Ministry of Health, this project introduces new method for curative non-invasive local treatment of head and neck cancers into practice in the Czech Republic. This state-of-the-art highly precision FMISO-image guided radiation treatment represents a method of radiation therapy for delivering a significantly higher than standard dose of ionizing radiation within the same overall treatment time directly to the most radioresistant hypoxic targets with maximal precision and effort to overcome the radioresistance of these tumor tissues while maintaining the same level of protection of surrounding healthy tissues as with standard treatment thus no compromising the dose constrains of surrounding organs and healthy tissues.

While available evidence confirmed efficiency and feasibility of this above-mentioned FMISO guided radiotherapy method, the usage of this method is still limited and rather experimental in the world. Indeed, there is no report from Czech authors in journals with the impact factor that is focused on this issue. The proposed project will consider feasibility of FMISO image guided increased dose definitive radiation therapy of head and neck cancers in conditions of the three large Czech comprehensive cancer centers and will evaluate its efficiency and toxicity.

Currently, there is a dramatic increase in development of new treatment possibilities for cancer patients. Treatment with the novel targeted therapy or immunotherapy is expensive (financial toxicity) and represents the important portion of money released for the treatment of oncology patients by health care insurance companies not only in the Czech Republic and subsequently raises the cost – benefit ratio questions. The current state-of-the-art radiotherapy remains the cornerstone of head and neck cancers treatment with proven efficacy. This novel proposed method would even increase the efficacy of the treatment of these malignancies with minimal added expenses.

The final consequence is the optimization of treatment from the patient´s perspective (personalized medicine).

Expected results and outputs of the project

We anticipate 2 principal primary results (2x Jimp – publication in international peer-reviewed top-rank journal with impact factor in the field of radiation oncology, oncology, head and neck cancers or nuclear medicine and 2 secondary results (1x Jsc and 1x Jost).

Currently, the radiotherapy of head and neck cancers with FMISO PET/CT guided increased dose is not yet provided worldwide routinely, but has the ability to be the practice changing treatment of these malignancies. Generally, the results of research conducted on such a comprehensive and available radiotherapy and imaging systems all three participating centers are equipped may have a serious impact on future daily practice. Another important output of our project is to employ young scientist (Ph.D. students or postdocs) into research projects.

Ethical issues

The statement of Institutional Review Boards regarding this research proposal are included in the final proposal form. Patient will be informed about all pros and cons of radiotherapy with increased dose. All the data collected will remain confidential and will be disclosed only with patient´s permission. Patient's identity will be protected to the extent permitted by the Czech Republic law. When the results of research will be published or discussed in conferences, no information will be included that would reveal patient's identity. The project and Informed Consent had been discussed with Ethical Committees in all three centers involved - Institutional Review Boards of Faculty Hospital Olomouc, Faculty Hospital Ostrava and Masaryk Memorial Cancer Institute.

Risk management and quality assurance

The primary methods of protection against risks are the careful development and implementation of study procedures by fully trained and supervised personnel. The rigorous application of inclusion/exclusion criteria and the local standards of care are designed to both detect evidence of and protect against adverse effects. The important issue is that all prepared radiotherapy treatment plans

will undergo rigorous central review process to minimize interobserver variabilities further ensuring unity in treatment volumes and homogeneity of treatment plans.

We identified these risks in addition to those mentioned in the Ethical issues statement:

- *Performance of FMISO PET/CT*. Common difficulties with the production of PET tracers or PET camera are considered as an unpredictable and in that case, patient will be excluded from the study in order to avoid possible delay in the initiation of radiation treatment.
- *Compliance of patients to control evaluations and questionnaires completeness*. This risk will be evaluated in the regular investigator´s meetings and appropriate steps will be performed to motivate patients remain in the study after last radiotherapy session (for example strict unification of visits dates to visits in another outpatient service).
- *Technical issues of Increased dose radiation therapy* all three participating departments of radiation oncology are equipped by at least two identical linear accelerators, so continuing treatment is ensured in the case of machine break or planned periodic maintenance inspections.

Competence of the applicant and the workplace and Information on preparedness of cooperating departments:

The participating centers have a preliminary experience with precise increased dose radiation therapy of head and neck cancers, the use of FMISO and adaptive radiotherapy. (40,41)

All involved centers have the equipment to ensure the most advanced radiotherapy of tumours of the head and neck (CT simulation verification system, linear accelerators with cone beam CT, systems of quality assurance in radiotherapy and other) and the most sophisticated methods of radiotherapy are involved for this group of patients - the use of highly conformal techniques as IMRT (intensity modulated radiotherapy) and VMAT (volumetric arc therapy with Jaw tracking). IGRT with on board cone beam CT and 6 degrees of freedom couch safely enables the application of simultaneous integrated boost dose escalation in high risk areas without increasing overall treatment time. These highly specialized techniques allow administrating high doses of radiation with substantial sparing of surrounded tissues. All three centers provide this treatment for the area of the Moravian region, but because of the good reputation of these centres, patients from all over the Czech Republic are frequently treated, too. Treatment of patients with head and neck cancers is widely implemented in all three involved centers. Physicians are integral parts of regional multidisciplinary teams for these tumours and the treatment regimens is always “up to date” employing currently most advanced treatment options and techniques. In addition to the challenging primary treatment a supportive treatment is secured adequately - dental care, nutritional care and treatment of acute radiation reactions of the skin and mucous membranes, analgesia and regular otorhinolaryngology examinations during radiation therapy if required.

All involved centers have PET/CT scanners. The harmonisation of measurements from different PET/CT imaging sites will be guaranteed by proper tuning of reconstruction parameters of each PET/CT scanner.

The Department of Oncology of Olomouc with the Chief of radiation therapy being also the lead investigator of the current project belongs between the most important cancer care centers in the Czech Republic. Currently, thanks to the modernization of technical equipment, it is one of the best-equipped radiotherapy departments in the Czech Republic, including the most modern available software for planning, deformable registration and online intrafraction correction of patient settings. Apart of providing health care it is also the center of excellence science and education in Oncology. It covers and organizes The best of ASTRO regularly for last 5 years. The department also has a strong background in medical oncology and internal medicine which is important for supportive care of patients with head and neck cancer who commonly have internal comorbidities.

The department is participating in numerous clinical trials and has strong publication record with over 20 papers published yearly in journals with impact factor, including top journals like New England Journal of Medicine or Lancet Oncology.

The importance of *Department of Oncology in Ostrava* exceeds the borders of the Czech Republic. It is well known worldwide for its “Cyber Knife” facility which is the most occupated Cyber knife in the world and many foreign patients are treated in the clinic. In addition to this device, the clinic is of course endowed with 2 linear accelerators. The clinic belongs between the mainstays in the treatment

of head and neck cancers in the Czech Republic for years with emphasis also on radiobiology. It is also known for its save implication of stereotactic boost on residual disease in nasopharyngeal cancer treatment with proof of its safety and efficacy.

The Masaryk Memorial Cancer Institute (MMCI) is both a medical facility and research institution established especially for the purpose of providing health care services and research in the areas of prevention, diagnosis and treatment of solid cancers. MMCI is an internationally accredited institute. Every year, approximately 3,500 new patients for radiotherapy are appointed, of those more than 200 a year are reffered for head and neck cancer treatment.
Department of radiation oncology at MMCI is the biggest radiotherapy department in the Czech Republic and centre of competence of IAEA with five linear accelerators including two specialized stereotactic devices Varian TrueBeam STx, version 2.5 quality assurance system. It is known as the leader in stereotactic radiotherapy pioneering stereotactic approaches in both intra- and extracranial stereotactic irradiation within the Czech Republic. It has more than 6 decades of clinical experience with head and neck cancer radiation treatment. The clinic of radiation oncology closely cooperates with two Otorhinolaryngology Clinics of University Hospitals in Brno and thus forms one of the largest Head and Neck cancer centers in the Czech Republic. The successful scientific cooperation in the field of head and cancers is established also with CEITEC - Central European Institute of Technology of Masaryk University.

Co-operation of the proposer with foreign research institutes
MMCI represents the Czech Republic in the OECI (Organisation of European Cancer Institutes) and is the only one Czech center certified as "Clinical Cancer Center OECI". The department of Radiation Oncology is acknowledged as IAEA Centre of competence.
Based on a long-term collaboration between the Masaryk University, Faculty of Medicine and the Mayo Clinic, Rochester, MN, USA, the team member, Tomas Kazda M.D., Ph.D. worked as a research fellow at the Department of Radiation Oncology, Mayo Clinic. Currently, the collaboration between these two institutes is still ongoing in terms of future research strategies and projects preparation.
In 2019, the Co-investigator Marek Slavik M.D., Ph.D., and a team member Tomas Prochazka MSC and in 2018 another team members, Petr Burkoň, M.D., Ph.D. and Tomáš Kazda, M.D., Ph.D. finished observational clinical fellowship at The James Cancer Hospital and Solove Research Institute, Ohio State University, Columbus, USA, Department of Radiation Oncology under supervision of prof. Chakravarti.

Faculty Hospital Ostrava is a member of StopStorm consortium (Jakub Cvek is national coordinator and international co-lead of one of eight work packages), this project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 945119.

Characteristics of the leading researchers in the team:
Associate Professor Martin Doležel, M.D., Ph.D., principal investigator of the project (51 papers in total, 21 as a first or corresponding author, 36 in journals with IF /15 as a first or corresponding author/, 11 pedagogic papers, WOS H-index 10) belongs to leading specialists working on various issues in the field of radiation treatment including head and neck cancers, gynecological malignancies and brachytherapy, is the President of Society for Radiation Oncology, Biology and Physics of the Czech Republic.
He is the head of radiation oncology department in Palacký University and University Hospital Olomouc, President of "Best of ASTRO Czech Republic", Local Manager of 2016 ESTRO Annual meeting in Wienna and 2020 ESTRO Teaching course on Clinical Practice and Implementation of IG-SBRT in Prague. He participated as a principal investigator or coprincipal investigator in many clinical studies dealing with treatment of many cancer types. His pioneering works consisted of implementation of MRI-based Image Guided Brachytherapy for Cervical cancers in the Czech Republic, integration of simultaneous integrated boost in treatment of prostate cancer, invention of audiovisual navigation in deep inspiration breath-hold radiotherapy and systematic implementation of

adaptive radiotherapy in head and neck cancer treatment. There are many scientific interests in his scope, currently the topic of Head and Neck cancers and adaptive radiotherapy is of much importance.
He will carry out consulting services for all co-investigators and as a leader will be responsible for project management and will provide methodology supervision. As a radiation oncologist he will provide radiation therapy management for the patients enrolled into the study, will also cooperate with medical physicists / dosimetrists and radiation therapists during the preparation of treatments plans and during irradiation itself. He will also participate in the evaluation of the treatment plans as a member of central review board, further on evaluation of results and their presentation and publication.

Associate Professor Jakub Cvek M.D., MSC, Ph.D.co-investigator and a deputy of principal investigator (35 papers in total, 19 as a first or corresponding author, 21 in foreign journals, 12 with IF, 7 pedagogic papers, WOS H-index 7) is the head of Oncology Clinic of University Hospital in Ostrava. He is Scientific Secretary of Society for Radiation Oncology, Biology and Physics of the Czech Republic
His main clinical interests are Head and Neck cancers and Stereotactic Ablative Radiotherapy, New radiotherapy techniques, and Radiobiology. Apart from other important publications he is the one of two authors of the most respected book on radiobiology ever published in the Czech Republic.
He will cooperate with the principal investigator in leadership and cooperation of the research team.
He will also provide methodology supervision and consultation activities. As a radiation oncologist he will provide radiation therapy management for the patients enrolled into the study, will also cooperate with medical physicists / dosimetrists and radiation therapists during the preparation of treatments plans and during irradiation itself. He will also participate in the evaluation of the treatmennt plans as a member of central review board and in the evaluation of results and their presentation and publication. He will also participate in the evaluation of results and their presentation and publication. He will be also responsible for clinical data database updates.

Marek Slávik, M.D., Ph.D., co-investigator of this project, (23 papers in total, 5 as a first or corresponding author, 14 in foreign journals, 14 with IF, 6 pedagogic papers, WOS H-index 4) is an assistant professor at Faculty of Medicine, Masaryk University. He works as the radiation oncologist and researcher at the Department of radiation oncology at MMCI. He is Department vice-head for research and development. He was awarded for his research in gastric cancer by Czech Society for Radiation Oncology, Biology and Physics in 2015. His main clinical interests are Head and Neck cancers and Stereotactic Ablative Radiotherapy, New radiotherapy techniques, and Radiobiology. He attended a lot of ESTRO teaching courses. He has studied SBRT techniques in Oslo University Hospital, Norway. At observational clinical fellowship at The James Cancer Hospital and Solove Research Institute, Ohio State University, Columbus, USA, he especially dealt with the issue of Head and Neck cancers.
He is a member of Head and Neck Committee in MMCI and in the two University Hospitals in Brno. As a member of research team he has been participating in various clinical studies and grant projects of which the successful grant project related to Head and Neck Cancer and Biomarkers of Tumour Hypoxia and MicroRNAs is of particular importance. Alongside it was his main Ph.D. thesis. He was responsible for the clinical part of the project and provided a smooth cooperation of participating researchers from different institutes. In the current project, he will provide methodology supervision and consultation activities. As a radiation oncologist he will provide radiation therapy management for the patients enrolled into the study, will also cooperate with medical physicists / dosimetrists and radiation therapists during the preparation of treatments plans and during irradiation itself. He will also participate in the evaluation of the treatmennt plans as a member of central review board and in the evaluation of results and their presentation and publication. He will be also responsible for clinical data database updates.

Member of the team Professor Bohuslav Melichar M.D., Ph.D., (350 papers in total, 140 as a first or corresponding author, 315 in foreign journals, 320 with IF, over 13 000 citations, WOS H index 48). Professor Melichar is the head of the Department of Oncology, Palacký University and University Hospital Olomouc. Besides medical oncology, he has also specialization in radiation oncology and

internal medicine, and his primary research interests are biomarkers and immunotherapy. He will be responsible for coordinating the supportive care of the patients.

Member of the team Associate Professor Pavel Koranda M.D., Ph.D. – is the chief of the Department of Nuclear Medicine Palacký University and University Hospital Olomouc, will be responsible for the performance of 18F-MISO PET/CT according to the protocol and will collaborate in the definition of target volumes guided by PET imaging.

Member of the team Tomáš Kazda, M.D. - is an Associate Professor at Faculty of Medicine, Masaryk University and radiation oncologist and researcher at the Department of radiation oncology at MMCI His research training was realized at Mayo Clinic, Rochester, Minnesota, USA, where he worked as a research fellow in the Department of radiation oncology. Currently, he also works as Director for Research and Development at Masaryk Memorial Cancer Institute. His research was repeatedly awarded by Czech Society for Radiation Oncology, Biology and Physics. In the current project he will provide a independent supervision of the project, will participate in the results evaluation and their presentation and publication.
Team leaders will meet regularly on the partners meetings where the decisions will be taken every half year unless otherwise arranged based on actual situation. Outputs of all meetings will be summarized in protocols for a later control, clear statements and for the evaluation process of the project, the results, deliverables and according to the state define the appropriate tasks and changes of the project.

Member of the team Tomas Prochazka, MSC. – radiation physicist, member of the auditing comitee of Society for Radiation Oncology, Biology and Physics of the Czech republic and the succesfull participant of the international radiotherapy planning challenges (e.g : QADS TG244 H&N Plan Study and many others, usually in first 10%-20% worldwide, title best perfomer) will be responsible for radiotherapy planning, independent control and verification of treatment plans. He will be also a member of central review board and participate in the evaluation of the treatment plans. He will participate in the results analysis and their presentation and publication.

Zdenek Rehak, Assoc. Prof. M.D., Ph.D. is the chief of the Department of Nuclear Medicine, MMCI. His research interests are PET/CT imaging, non-FDG PET tracers, and new clinical applications of molecular imaging. His research was awarded by the Czech Society of Nuclear Medicine, Czech Society of Rheumatology, Czech Society of Internal Medicine (3x best publication).
For patients included at MMCI, he will be responsible for the performance of 18F-MISO PET/CT according to the protocol and will collaborate in the definition of target volumes guided by PET imaging. He will also participate in the evaluation of results and their presentation and publication.

Member of the team Martin Havel, Ph.D.MD. – nuclear medicine specialist, will be responsible for the performance of 18F-MISO PET/CT according to the protocol and will collaborate in the definition of target volumes guided by PET imaging.

Radiation oncologists will provide radiation therapy management for the patients enrolled into the study, will also cooperate with medical physicists / dosimetrists and radiation therapists during the preparation of treatments plans and during irradiation itself
Medical physicist, will be responsible for stereotactic radiotherapy planning, independent control and verification of treatment plans. He will participate in the results analysis and their presentation and publication.
The Radiation Therapist will be responsible for patients' education and coordination of treatment in treatment units and collections of case report forms.
The Nurses will be responsible for patients' education and coordination of follow up, collection of case report forms.
The Study Coordinators will cover all administrative paperwork and will participate in the coordination of workflow. They will be also included in clinical data database management (collection of case report forms), communication with patients and organization of research team meetings.

All members of the research teams will be included in interim analysis and will take part at regular meetings.

FMISO-based protocol for adaptive radiotherapy in head and neck cancer

The radiotherapy protocol will include two dose-escalation regimens. The dose in hypoxic tumor volume will be escalated either by conventional RT or stereotactic radiotherapy technique. Concurrent chemotherapy cisplatin will be administered weekly 35-40 mg/m2 or every three weeks 80-100 mg/m2. The parameter of cumulative cisplatin dose of 200 mg/m2 during the whole course of radiotherapy will be also taken into account. Patients will be examined and monitored at least every two weeks.

Inclusion Criteria:

- Has a pathologically proven new diagnosis of oropharyngeal p16 negative, or larynx/hypopharynx/oral cavity (independent of p16) squamous cell carcinoma III, IV clinical stage
- Has evaluable tumor burden assessed by computed tomography scan or magnetic resonance imaging, based on RECIST (Response Evaluation Criteria in Solid Tumours) version 1.1
- Is eligible for definitive chemoradiation or hyperfractionated accelerated radiotherapy and not considered for primary surgery based on multidisciplinary tumor board decision - prior surgical debulking, including tonsillectomy, for the head and neck cancer under study is not allowed.
- Has Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 performed within 10 days prior to receiving the first dose of study therapy
- Adequate kidney and liver function

Exclusion Criteria:

- Has cancer outside of the oropharynx, larynx, and hypopharynx or oral cavity, such as nasopharyngeal, sinus, other para-nasal, or other unknown primary head and neck cancer
- Has had prior systemic therapy, targeted therapy, radiotherapy treatment or radical surgery for head and neck cancer
- Has known active Hepatitis B or C
- Has known history of Human Immunodeficiency Virus (HIV)
- Has history of a diagnosed and/or treated hematologic or primary solid tumor malignancy, unless in remission for at least 5 years prior to randomization
- Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis
- Has had previous allogeneic tissue/solid organ transplant
- Has active infection requiring systemic therapy

FMISO imaging procedure

The extent of the field to be examined on 18F-MISO PET/CT will be determined by imaging of malignant tissue on FDG PET/CT. The studies published so far differ partly in the way the hypoxic area is delineated. Most often, the target-muscle ratio 1.2 or 1.4 is used to define the boundaries of the hypoxia area. The researchers decided to use in this project the more commonly used value of 1.4 in this study.

The multicentre study using 18F-FDG and 18F-MISO scans will be conducted at 3 PET/CT sites equipped with different PET/CT scanners (two with LSO scintillators – Siemens Biograph mCT 40 and Siemens Biograph mCT flow, one with BGO scintilators GE Discovery IQ). The harmonisation of measurements from different PET/CT imaging sites will be guaranteed by proper tuning of reconstruction parameters of each PET/CT scanner. The target reconstruction settings must fulfil the EARL (Evaluation and Report Language) version 1 criteria. Body phantom and procedure described in https://ejnmmiphys.springeropen.com/articles/10.1186/s40658-019-0257-8 will be used to generate RC (recovery coefficients).

Treatment preparation (radiotherapy planning procedures):
Patients will follow standard radiotherapy planning procedures. Supine body position and head fixation with 5 point thermoplastic mask (Orfit). Contrast-enhanced planning CT with 3 mm slice thickness will be performed at the initiation of treatment planning. A new, current contrast-enhanced planning CT will be repeated before the initiation of the stereotactic hypofractionated boost. Except for these scheduled examinations a new planning CT scan will be repeated individually in case of weight loss, anatomical changes happening during the treatment or rapid tumour regression and soft tissues changes. Additionally planning MR examination could be performed for better target volume determination and soft tissue imaging.
As part of the RT planning process, FMISO PET/CT will be used to an accurate definition of hypoxic areas within the tumor volume. PET scans will be registered with planning CT during radiotherapy planning. FMISO PET/CT will be repeated after the 11th fraction of treatment, respectively.

Target volumes and dose and fractionation:
Definition of gross tumor volumes (GTV), clinical target volumes (CTV) and planning target volumes (PTV) will follow recommendations of DAHANCA, EORTC and RTOG guidelines.

The conventional radiotherapy protocol:
Standard fractionation regimen: 70 Gy/54 Gy in 33 fractions

GTV primary - CTV - PTV (5+5mm): for dose 70 Gy in 33 fractions
GTV LN bulky (> 3cm) - PTV (5mm): for dose 70 Gy in 33 fractions
LN low risk (for elective irradiation) - CTV - PTV (3mm-5mm): for dose 54 Gy in 33 fractions

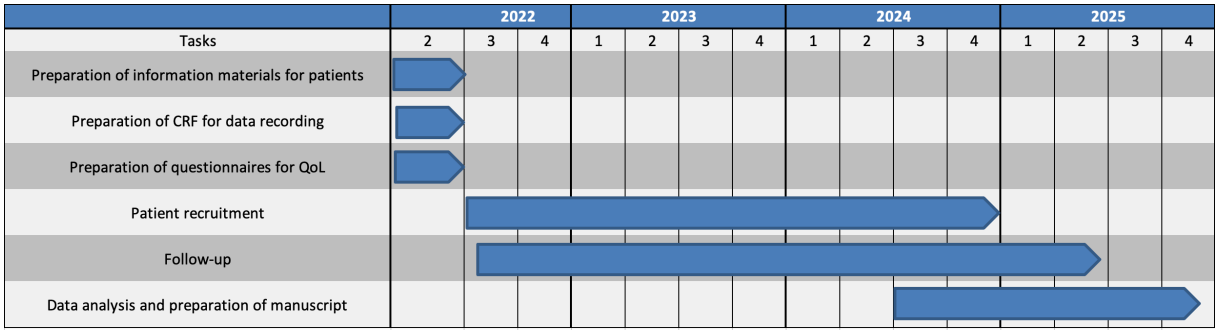
Dose escalated radiotherapy protocol:
Dose escalated radiotherapy protocol: 75,9 – 79,2 Gy in 33 fractions

GTV hypoxic or any hypoxic LN > 2cm - PTV (0mm): dose 75,9 – 79,2 Gy in 33 fractions
(Contours must be subtracted and reduce by 3mm in case of close relation to the skin, bones or large blood vessels)
GTV primary - CTV - PTV (5+5mm): for dose 70 Gy in 33 fractions
LN low risk (for elective irradiation) – CTV - PTV (3mm-5mm): for dose 54 Gy in 33 fractions

Radiotherapy technique:
The conventional radiotherapy protocol will use IMRT or VMAT technique with Simultaneous Integrated Boost. IGRT (Image-guided radiation therapy) will be used daily (cone-beam CT) with online registration and patients position correction by RTTs. Subsequent offline evaluations will be performed by the radiation oncologist.
Toxicity management and scoring:
The Common Terminology Criteria for Adverse Events (CTCAE) scoring systém version 5.0 will be used to evaluate acute and late radiation toxicity. Patients will be examined whenever their condition worsen but no longer than in 2 weeks. Each patients review will include a blood test (blood count, biochemistry, inflammatory markers). Percutaneous endoscopic gastrostomy tube placement will be indicated in patients with weight loss of > 10% body weight before or during RT or presence of abnormal swallowing and severe pain and multiple comorbidities.
Evaluation of treatment response:
Evaluation of treatment response during follow-up will be based on clinical examination and imaging examination according to the guidelines recommendations. Twelve weeks after finishing the radiotherapy a new restaging PET-CT will be performed to exclude the persistence of the residue or tumor progression. In the case of partial regression, the PET-CT will be repeated in 12 weeks. A similar approach will be preferred in the case of complete regression.
Quality of life scoring:
Quality of life will be assessed according to the EQ D5 questionnaire

Follow-up:
HaP exam: Year 1: every 3 months
Year 2: every 2-6 months
Year 3-5: every 4-8 months
After 5 years: every 12 months
TSH: every 12 months

Time schedule



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