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Understanding Toxicity of Thoracic Radiation Therapy – The Influence of Immune Checkpoint Inhibitors and Alpha-2-Macroglobulin

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Understanding Toxicity of Thoracic Radiation Therapy – The Influence of Immune Checkpoint Inhibitors and Alpha-2-Macroglobulin

Abstract

Radiation therapy (RT) is essential in the treatment of thoracic malignancies. New challenges arise from the combination of radiation therapy (RT) and immune checkpoint inhibitors (ICI) as well as the identification of radioprotectors and predictive factors for adverse events (AE). In a retrospective study, data on common AEs was collected for 79 patients having received both thoracic RT and ICI therapy. Grade ≥ 2 pneumonitis, esophagitis, and dermatitis rates were 6.3, 7.6., and 10.1%, respectively. No differences in AE rates were seen whether the treatment timing was concurrent, closely timed (≤ four weeks interval), or sequential (> four weeks to six months interval). For the alpha-2-macroglobulin (α2M) study, 258 patients that received thoracic radiation for any kind of malignancy had pre-treatment serum α2M levels measured. A2M, which has shown radioprotective effects in preclinical studies, as well as a range of clinical and dosimetric factors were included in predictive models to analyze their association with the development of radiation pneumonitis and esophagitis. Grade ≥ 2 radiation pneumonitis and esophagitis rates of 14 and 23.6% were reported. The mean α2M level was 217.3 mg/dl in current smokers compared to 207.3 and 185.4 mg/dl in former and never smokers. Final predictive models included D65 in lung, max dose in heart, and treatment days for pneumonitis as well as D25, D40, and treatment days for esophagitis. Our studies showed no significant increase of common AEs after thoracic RT and ICI therapy compared to RT or ICI alone and no difference of AE rates with different treatment timing intervals. Although pre-RT levels of α2M did not improve the predictive power of our models for pneumonitis, esophagitis, and dermatitis, we found a univariate association between α2M levels and radiation esophagitis as well as the smoking status of the patients. Furthermore, we were able to validate several clinical and dosimetric factors with our predictive models.

Key Words

Thoracic radiation therapy, immunotherapy, immune checkpoint inhibitor, toxicity, adverse event, alpha-2-Macroglobulin

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List of Abbreviations

3D-CRT	3D conformal radiation therapy
4DRT	4D radiation therapy
α2M	Alpha-2-Macroglobulin
ADL	Activity of daily life
AE	Adverse event
ALK	Anaplastic lymphoma kinase
APC	Antigen-presenting cell
ASCO	American Society of Clinical Oncology
AUC	Area under the curve
BAL	Bronchoalveolar lavage
CERR	Computational Environment for Radiological Research
CI	Confidence interval
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
cGy	Centigray
dL	Deciliter
DVH	Dose volume histogram
Dx	Dose
EGFR	Epidermal growth factor receptor
fD	Fractional dose
FDA	U.S. Food and Drug Administration
GTV	Gross tumor volume
Gy	Gray
ICI	Immune checkpoint inhibitor
IMRT	Intensity-modulated radiation therapy
irAE	Immune-related adverse event
LASSO	Least absolute shrinkage and selection operator
mg	Milligram
MLC	Multi-leaf collimator
MLD	Mean lung dose
MnSOD	Manganese superoxide dismutase
MPM	Malignant pleural mesothelioma
MSKCC	Memorial Sloan Kettering Cancer Center
NSCLC	Non-small cell lung cancer
NTCP	Normal tissue complication probability
OS	Overall survival
PFS	progression-free survival
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PRO	Patient-reported outcome
RET	Rearranged during transfection gene
ROS1	c-ROS oncogene 1
Rs	Spearman’s correlation

RT	Radiation therapy
RTOG	Radiation Therapy Oncology Group
SABR	Stereotactic ablative radiation therapy
SBRT	Stereotactic body radiation therapy
SCLC	Small cell lung cancer
SD	Standard deviation
SNP	Single nucleotide polymorphism
TCP	Tumor control probability
TCR	T-cell receptors
TGF	Transforming growth factor
TNF	Tumor necrosis factor
Treg	Regulatory T cell
U.S.	United States
VMAT	Volumetric modulated arc therapy
Vx	Volume

1. Study Aim

Radiation therapy (RT) holds a central role in the treatment of thoracic malignancies and metastases found in the thorax. RT is generally viewed as a very tolerable and safe treatment option, especially in older patients and patients with comorbidities. Considering the aging population worldwide and the resulting influx of cancer patients belonging to aforementioned categories, the significance of RT is continuing to rise. In this context, toxicity analysis and prevention has become a core aim in RT research, alongside heightening efficacy.

A further challenge arises from the fact that thoracic RT is often implemented in a multi-modality treatment setting, thus adding toxicity from other therapies to the equation. During most part of the past century the main combination partner to consider was chemotherapy, especially platinum-based regimens used in lung cancer treatment. The last two decades however have introduced a new, significant group of drugs into oncotherapy and, as a result, a new factor to the "toxicity equation": immune checkpoint inhibitors (ICI). First studied and approved in patients with malignant melanoma, checkpoint inhibitors have since shown promising results in the setting of advanced non-small cell lung cancer (NSCLC). While there is mounting evidence that the combination of RT plus ICI can yield survival benefits for certain cancer patients, planning of clinical studies, especially in a prospective setting, is often delayed by the fear of additive toxicity rates. To alleviate these concerns, research should focus on data which are available at the time. Thankfully, while combination treatments of RT plus ICI have only just advanced to the prospective clinical study phase in the last few years, there is already over a decade worth of trials of immune checkpoint inhibitors in thoracic oncology, especially in one of the largest groups in this field: patients with advanced NSCLC. The aim of the first study presented in this thesis was of a descriptive nature. Using data collected from patients having received both RT and ICI during their treatment, the goal was identifying adverse events (AE) which occurred after the second of both treatment modalities had been started. As there was yet insufficient data on the optimal treatment timing when combining RT and ICI, this study's inclusion criteria included a cutoff value of six months for the maximum time period between both treatments and analyzed AEs registered both in a concurrent and a sequential treatment mode. The goal at the time was providing a statistical foundation for the clinical observation that patients receiving both therapies for thoracic malignancies did not seem to suffer from significantly more toxicity than was to be expected from either therapy alone.

Most research advances in the field of radiation-induced toxicity stem from improved radiation technique and a better understanding of the clinical and dosimetric factors correlating with higher toxicity rates. Given the technological improvements in radiation treatment planning and the digitalization of health data, predictive modelling using large amounts of data has become a convenient and precise method to analyze the significance of said factors in the development of RT-related adverse events. Dosimetrics are readily available for all treatment plans and can, to a certain extent, be adapted to meet established dose thresholds while planning. Although clinical factors like pre-existing conditions and baseline organ function have been thoroughly investigated for their effects on irradiated tissue, little progress has been made identifying intrinsic factors or biomarkers that may be harnessed to prevent or milden the development of radiation toxicity. To date, only few agents have been found that have a proven mitigating effect on radiation toxicity and even fewer have shown positive effects in a clinical setting. A protein which has demonstrated radioprotective effects in animal studies is alpha-2-Macroglobulin (α2M). In the second study of this thesis, treatment and clinical data was gathered in patients having received thoracic radiation. A predictive model was built analyzing the influence of pre-RT human serum α2M levels on the development of three common adverse events in thoracic radiation: pneumonitis, esophagitis, and dermatitis. In addition, the predictive model included a range of dosimetric factors as well as patient-specific factors like smoking history.

Lung cancer, the primary therapeutic target of thoracic radiation, remains one of the most common and aggressive malignancies worldwide. Given the high sensitivity of healthy lung tissue and other intrathoracic organs to radiation, the systematic analysis and prevention of adverse events in patients receiving thoracic RT is of utmost importance in the field of thoracic radiotherapy research. The following two studies aims at providing some results in two key research areas:

Can we safely combine thoracic radiation therapy with new immune checkpoint inhibitors?
and
What intrinsic factors contribute to the development of thoracic radiation-induced toxicity?

2. Introduction

2.1. Thoracic Malignancies

The term thoracic malignancy most commonly includes cancers of the lung, pleura, thymus, and heart. Examples include non-small and small cell lung cancer, malignant pleural and pericardial mesothelioma, thymoma, and thymic carcinoma. When referring to treatment options, especially radiation therapy, this definition may be expanded to include metastases of other malignant diseases which have spread to the lung or pleura.

Lung cancer is the most common cancer type worldwide, accounting for 13% of all cancers. It is the malignancy resulting in the most cancer deaths worldwide (20 %) [1]. Smoking remains the foremost risk factor for developing a tumor of the lung. Changes in smoking habits have led to a convergence of incidences in men and women but also to a slight decline of incidence in men globally. A similar development in women has, until now, only been observed in few countries including Australia and the US [1]. 95% of lung cancers are classified as either non-small cell, including histological subtypes such as adenocarcinoma and squamous cell carcinoma, or small cell lung cancer (SCLC), which belongs to the group of neuroendocrine tumors. Non-small cell lung cancers (NSCLC) make up the largest part of lung cancer cases (around 85%), with adenocarcinoma being the predominant histology in patients that are younger and never-smokers. SCLC occurs almost exclusively in patients that have a history of smoking. In recent years, developments in diagnostics and therapeutics have led to a shift in lung cancer classification, which increasingly relies on immunohistochemical analysis and the identification of driver mutations as determinants for therapeutic decisions [2].

Malignant mesothelioma is a disease which can develop in the mesothelium lining of the pleural and peritoneal cavity, pericardium, and tunica vaginalis. 80% of mesothelioma cases are of pleural origin. Prior exposure to asbestos has been identified as the main risk factor, accounting for over 80% of cases in males [3,4]. Due to a mean latency of forty years between exposure and development of malignant pleural mesothelioma (MPM), the risk factor attribution may be difficult in patients with no known occupational exposure to asbestos [5]. The incidence of MPM, which increased heavily since the 1960s, has been predicted to peak between 2010 and 2020 in European countries due to the prohibition of asbestos [6] and may have peaked already in countries like the USA and Sweden [7,8]. Nevertheless, production and

use of asbestos persists in large countries like Russia, China, Brazil, and India, meaning the global burden of asbestos-related disease will most likely continue to rise for some time.

Pulmonary metastases are common in many types of cancer, among them malignant melanoma, sarcoma, and tumors of the breast, colon, kidney, testicle, uterus, and head and neck [9]. In contrast to patients without known malignancy where solitary pulmonary lesions are far more likely to be benign than of malignant origin, studies have shown that around 25% of solitary pulmonary nodules in patients with extrathoracic primaries are metastases [10].

2.2. Treatment

2.2.1. Thoracic Radiation Therapy

Development of thoracic radiation therapy techniques has been mainly driven by advances in lung cancer treatment, as these present the largest fraction of thoracic malignancies. When used to treat unresectable locally advanced or medically inoperable lung cancers, the goal of radiation therapy is to provide durable local control while simultaneously avoiding too much radiation-induced toxicity to the surrounding normal tissue. In thoracic radiation therapy, special care must be given to spare sensitive organs like the heart, spinal cord, and healthy surrounding lung tissue from high doses of radiation.

Dosage and fractionation have been among the most common parameters under investigation in search of an optimal radiation regimen. Standard fractionation has developed to be defined as 1.8 to 2.75 Gy in 25 to 40 fractions amounting to five to eight weeks of treatment with a total dose of 55 to 75 Gy [11]. Variations include hypo- and hyperfractionation as well as accelerated regimens. An important development, especially for early-stage lung cancer, which was first reported in the mid-1990s was stereotactic body radiation therapy (SBRT), also known as stereotactic ablative radiation therapy (SABR). SBRT typically consists of one to five fractions of large RT doses (10 to 34 Gy) delivered over a one-to-two-week period. In numerous studies in the past 20 years, SBRT has been shown to be effective and safe in early-stage peripheral tumors [12,13], while high radiation doses for central tumors can quickly lead to excessive toxicity [14]. Currently used predominantly in patients who are medically inoperable, SBRT is being considered as an alternative to surgery in operable early-stage tumors in ongoing trials (NCT02468024, NCT02984761).

Advances in RT, including SBRT, over the past decades would not have been possible without computerized RT planning and widespread use of computer tomography imaging which led to the development of 3D conformal RT (3D-CRT). Using "beam's eye view" and calculating doses according to the patient's three-dimensional anatomy, RT can be planned more "conformal", i.e. sparing more healthy tissue while increasing dosage to the target volume [15]. A significant difficulty in treating thoracic tumors with RT arises from the fact that respiratory and cardiac motion must be accounted for while irradiating. Several techniques have emerged of the past decades which ultimately led to what is now described as 4-dimensional radiation therapy (4DRT). These techniques include respiratory gated RT (e.g. active breathing control [16] and deep inspiration breath hold [17]), external and internal fiducials to track tumor motion, and adaptation of computed tomography (CT) and positron emission tomography imaging to account for tumor motion. The advent of multi-leaf collimators (MLCs), replacing custom lead blocks used before, has facilitated the rise of new advances like intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT). In IMRT, complex modulation produces different radiation intensity across the radiation beam, making highly conformal target volumes possible [18]. VMAT uses a variation of gantry rotation speed, dose rate, MLC position, and collimator angle to achieve a similar goal as IMRT [19].

Radiation therapy alone is seldom sufficient in the treatment of lung cancer, especially in advanced stages. Beginning in the late 1970's, research began focusing on harnessing the additive potential of combining radiotherapy with systemic therapies like chemotherapy [20]. Since then, concurrent and sequential chemo-RT have become an essential part of multi-modality treatment in thoracic malignancies. Common cytotoxic agents used in combination are described below in the section 2.2.4. Standard-dose RT has proven most efficient and safe in chemo-RT regimens in locally advanced lung cancer [21]. In the advent of new systemic treatment options such as immune checkpoint inhibitors, questions have naturally arisen concerning the combination of such modalities with radiation therapy.

2.2.2. Immune Checkpoint Inhibitors

Immuno-oncology has been a rapidly growing area of research in the last two decades. As more and more mechanisms with which cancer cells evade the body's immune system are being investigated, so are ways to harness this knowledge and inhibit these "loopholes". Significant

evasion techniques which enable tumor survival and growth include attraction of immune suppressive cells into the tumor microenvironment, production of immune suppressive cytokines, downregulation of tumor antigen presentation, and induction of immune tolerance or deviation by suppressing costimulatory signals. So-called checkpoint inhibitors are monoclonal antibodies that can suppress either inhibitory receptors on immune cells or their corresponding ligands on antigen-presenting cells and tumor cells. Two checkpoint inhibitor targets that have gained the most traction in research and development are cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 / programmed death-ligand 1 (PD-1/PD-L1).

The PD-1 pathway has been identified as a crucial element in the regulation of autoimmunity as well as central and peripheral T cell tolerance [22,23]. PD-1 is a protein expressed on activated T cells but also on some regulatory T cells, B cells, and natural killer cells. Its ligands, PD-L1 and PD-L2, are found on both hematopoietic as well as non-hematopoietic cells and are also expressed by some tumor cells [24]. Binding of the receptor to one of its ligands inhibits several downstream signaling pathways, leading to a decrease of T cell activation and cytokine production, peripheral T cell exhaustion and a promotion of effector T cell to regulatory T cell (Treg) conversion, among other effects. By upregulating the expression of PD-L1 on their surfaces, tumor cells promote peripheral T-cell exhaustion and thus evade a heightened immune response. Studies in mice models and cell lines first demonstrated the anti-tumor potency of blocking the PD-1 checkpoint [25,26]. Since then, the development of immune checkpoint inhibitors has rapidly accelerated. Durable response at acceptable rates of adverse events was first shown in melanoma, NSCLC and renal-cell cancer [27]. The United States Food and Drug Administration (FDA) has currently approved nivolumab, pembrolizumab, cemiplimab, dostarlimab (PD-1 inhibitors), as well as atezolizumab, avelumab, and durvalumab (PD-L1 inhibitors) for the treatment of several solid tumors.

Although CTLA-4 has been found to play a significant role in the regulation of T cell-mediated immune reaction, there still is no consensus on its exact functions and properties decades after it was first discovered [28]. T cell activation relies on co-stimulatory signals in addition to the binding of T-cell receptors (TCR) to antigens presented by the major histocompatibility complexes (MHC) present on antigen-presenting cells (APCs). Co-stimulatory molecules such as CD80 and CD86 on APCs bind to CD28 molecules on T cells. CTLA-4, which is expressed by resting Treg cells and activated T cells, binds to the same molecules as CD28 but with a higher affinity, thereby competing over available binding molecules. In addition to suppressing

co-stimulation required for further activation of the T cell, CTLA-4 binding may also induce inhibitory signals [29,30]. CTLA-4 further impairs co-stimulation by capturing and removing CD80/CD86 from APCs via trans-endocytosis [31]. Following promising preclinical studies [32], anti-CTLA-4 antibodies have been successfully implemented in the treatment of cancer patients. Ipilimumab was the first checkpoint inhibitor to gain approval by the U.S. Food and Drug Administration (FDA) for the treatment of advanced melanoma, showing improved and durable overall survival (OS) in several studies [33]. A second anti-CTLA-4 antibody called Tremelimumab is still under investigation but has not attained approval in any setting.

2.2.3. Combination of Checkpoint Inhibitors and Radiation Therapy

The rise of a new therapeutic pathway offers a wide spectrum of combination treatments to be studied. With checkpoint inhibitors, research rapidly encompassed combinations of two or more antibodies (PD-1/PD-L1 + CTLA-4), as well as combinations of immunotherapy with chemotherapy and radiation therapy. First described as the “abscopal effect” in 1953 [34] and more recently in the 2000s, a growing body of evidence supports the concept of an immune-mediated systemic tumor response to radiation therapy at non-irradiated sites. Pre-clinical models demonstrated that a functioning immune system, specifically T-cells, is a prerequisite for the abscopal effect and that this effect may be harnessed by combining RT with immunotherapy [35,36]. Several case-reports and retrospective studies showed prolonged survival and systemic response in patients with metastatic melanoma, NSCLC, or renal cell cancer that often received palliative RT after having progressed on several lines of therapy including immune checkpoint inhibitors [37,38]. A secondary analysis of KEYNOTE-001, a phase 1 trial of pembrolizumab in patients with advanced NSCLC, added evidence by demonstrating prolonged progression-free and overall survival in patients treated with pembrolizumab that had previously received any kind of RT, including thoracic and cranial irradiation [39]. Until 2016, results from combination (chemo)-RT and immune checkpoint inhibitors in NSCLC were available solely in the context of retrospective studies or reports with limited possibilities of comparing RT and immunotherapy regimens or timing. In November 2017, first results of the PACIFIC trial, a phase III trial of durvalumab (anti-PD-L1 antibody) versus placebo as consolidation therapy after chemo-RT in patients with stage III NSCLC, were published. Progression-free survival (PFS) as well as overall survival was significantly longer with durvalumab compared to placebo and there were no significant safety

differences reported between both groups [40]. Although specifically combining RT and immunotherapy was not a primary goal or analysis endpoint of this study, survival and safety results in this study population, with approximately a quarter of patients being randomized to treatment (durvalumab versus placebo) within 14 days of completing RT, are promising [41].

2.2.4. Treatment Concepts

As is the case for most malignancies, treatment of thoracic tumors mainly builds on four oncotherapeutic pillars: surgery, chemotherapy, radiation therapy (RT) and targeted therapies like immune checkpoint inhibitors.

Non-Small Cell Lung Cancer

In early-stage NSCLC, surgical resection should be performed whenever feasible with the extent of resection depending on the size and location of the tumor. For patients who are medically inoperable or refuse surgery, options include RT (both conventional and stereotactic body radiation therapy), radiofrequency ablation, and cryoablation. Patients with resectable NSCLC have been shown to benefit from adjuvant chemotherapy, most commonly consisting of a platinum-based regimen [42]. Definitive concurrent chemo-RT is suggested as soon as there is evidence of mediastinal disease. Surgical resection can still be considered for certain patient with only limited involvement of the mediastinal lymph nodes. In this subset of patients, neoadjuvant chemotherapy or chemoradiation as well as postoperative RT should be considered as part of the multimodal treatment approach. For stage III patients that have not progressed on concurrent chemoradiation, Durvalumab, an anti-PD-L1-antibody, has been approved by the FDA for maintenance therapy following results from the PACIFIC trial [40].

In recent years, first line therapy for advanced NSCLC and many other cancer types has been increasingly determined by molecular testing. Testing in lung cancer includes the search for common driver mutations such as EGFR (epidermal growth factor receptor) and BRAF mutations, ALK (anaplastic lymphoma kinase), ROS1 (c-ROS oncogene 1), and RET (rearranged during transfection gene) rearrangements, and MET abnormalities as well as the expression levels of the PD-L1 ligand. FDA-approved first-line therapeutics are available if one of the abovenamed driver mutations is present, which has been shown to be the case in 50 to 64% of patients [43]. If no targetable mutation is found, initial therapy is based on the expression level of PD-L1. When expression is greater than 50%, it is recommended to begin

treatment with either pembrolizumab or atezolizumab monotherapy or combination immunotherapy and chemotherapy. If PD-L1 expression is lower than 50%, concurrent doublet chemotherapy and pembrolizumab is preferred, although pembrolizumab monotherapy is also FDA-approved in this setting. In addition to systemic treatment, localized therapy of distant metastasis, most commonly in form of palliative RT, may also play a significant role for the quality of life in patients with metastatic disease.

Small Cell Lung Cancer

Systemic therapy is central in the treatment of SCLC due to its fast growth and early development of metastases. In limited stage SCLC, defined as disease limited to the ipsilateral hemithorax and regional lymph nodes, the most common approach involves concurrent chemoradiation with etoposide plus carbo-/cisplatin. This is followed by prophylactic cranial irradiation in patients showing significant or complete response. For the small group of patients in which initial staging shows no signs of nodal disease, surgical resection may be considered, followed by adjuvant chemotherapy or, if pathology shows nodal involvement, chemoradiation.

Given the nature of this cancer type, most patients present with extensive stage disease, having spread beyond the ipsilateral hemithorax. The current first-line option in extensive stage SCLC is combination chemotherapy (etoposide plus carbo/cisplatin) and immune checkpoint inhibition using atezolizumab or durvalumab (both anti-PD-L1 antibodies). The addition of immunotherapy to chemotherapy followed by maintenance immunotherapy showed a significant survival benefit in two large, randomized studies compared to chemotherapy alone [44,45].

Malignant pleural mesothelioma

Upon diagnosis of malignant pleural mesothelioma, clinical staging is central to determine further disease management. In approximately 20% of cases, classified as clinical stage I to IIIA and displaying epithelial or biphasic histology, a surgical resection accompanied by (neo)adjuvant chemotherapy and RT may be recommended if the patient is medically operable. Surgery should only be performed in specialized centers and if the surgeons deem a macroscopically complete resection (R0 or R1) feasible. Definitive surgical options include extrapleural pneumonectomy, pleurectomy/decortication, and extended pleurectomy/ decortication. Hemithoracic RT can be delivered as IMRT in highly specialized centers.

Patients who completed tri-modality treatment showed a promising median overall survival of 20 to 29 months in several studies [46,47].

Unfortunately, most patients are diagnosed at a higher stage, at which the disease is determined to be irresectable. In October 2020, the FDA approved the combination of nivolumab and ipilimumab as first-line treatment in advanced MPM, based on results from the Checkmate 743 trial [48]. Before that, first-line therapy in these cases consisted of chemotherapy with cisplatin and pemetrexed, a regimen which showed superior OS compared to cisplatin alone in a phase III trial [49]. The addition of bevacizumab has been tested in several trials with mixed results but with one large phase III study showing benefits in OS and PFS [50]. There is no standard second- or third-line therapy in MPM. If progression occurs over six months after first-line therapy, options include a further round of a platinum/pemetrexed-based regimen or single-agent chemotherapy with pemetrexed. For earlier or multiple recurrences choices include immunotherapy (pembrolizumab, nivolumab (single-agent), and nivolumab with ipilimumab) or single-agent chemotherapy with gemcitabine, vinca alkaloids or anthracyclines.

2.3. Toxicities and Adverse Events

2.3.1. Assessment and Classification

When investigating new therapeutic procedures or drugs, a key variable in the analysis is the risk or the negative effects which a treatment carries. To adequately compare these risks, a framework of precise definitions is needed. In oncology research, such a framework was initialized by the National Cancer Institute. In 1982, they introduced the Common Toxicity Criteria (CTC) which aims at offering a comprehensive grading scheme for adverse events in oncology trials. Since its launch, several updated editions have been published, most recently in 2017. The Common Terminology Criteria for Adverse Events (CTCAE), as they are called since 2006, provide definitions for adverse events in 26 categories, each graded from 1 to 5. As the term “toxicity” does not have a clear definition, it is suggested that the term “adverse event” is used in the setting of medical documentation and scientific analysis. The CTCAE defines an Adverse Event (AE) as “*any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a*

medical treatment or procedure" [51]. Each AE is graded depending on severity based on a general guideline, which can be found in Table 1.

Table 1. Guideline for grading criteria of Adverse Events (CTCAE) [51]

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily life (ADL)
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death

Other frameworks for assessing toxicity include patient-reported outcomes (PRO), which have been standardized in the PRO-CTCAE measurement system [52] and are being implemented increasingly. Furthermore, guidelines which assess adverse events in relation to the benefits of a treatment, such as the European Society for Medical Oncology Magnitude of Clinical Benefit Scale [53] and the American Society for Clinical Oncology Value Framework [54] have been developed.

2.3.2. Thoracic Radiation Therapy

The challenge of radiation therapy lies in finding the balance between maximum local tumor control and minimal healthy tissue damage. To visualize this situation as seen in Figure 1, the tumor control probability (TCP) for any given tumor can be plotted as a sigmoid curve against the applied radiation dose. A second curve (ideally) to the right of the TCP curve represents the normal tissue complication probability (NCTP). A higher radiation dose corresponds with higher tumor control but also with a higher probability of tissue damage. The relationship between both these curves is known as the therapeutic ratio. Both curves can be influenced by different factors, thus changing the size of the therapeutic ratio. Improved radiation techniques like 3D-CRT and IMRT as well as radioprotectors can shift the NTCP curve to the right, while chemotherapy and other cell sensitizers can lead to a left-shift of both curves.

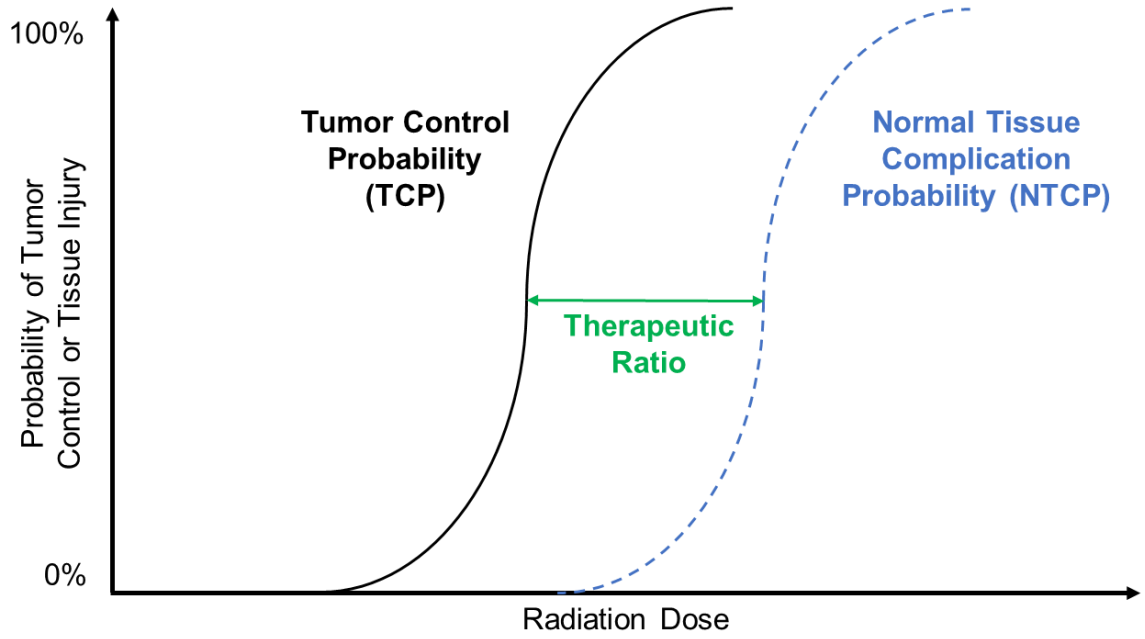


Figure 1. Relationship of Tumor Control and Normal Tissue Complication Probability

Toxicity caused by thoracic RT includes both location-specific adverse events like pneumonitis, esophagitis, and toxicity in heart and spinal cord, as well as adverse events associated with RT to any location like fatigue, hematological changes, and dermatitis in the radiation field. Bone marrow and spinal cord toxicity such as radiation myelopathy are not commonly seen with thoracic RT alone, as dose restraints to the spinal cord can usually be adhered to without much difficulty. However, hematological toxicity, especially myelosuppression, can become an issue when thoracic RT and chemotherapy are delivered concurrently [55].

Radiation induced lung injury can manifest as an acute or late toxicity. Acute radiation pneumonitis is an inflammation of lung tissue which is caused by a cascade of different proinflammatory and chemotactic cytokines, expressed after initial tissue damage and hypoxia caused by the radiation [56]. Although it can remain asymptomatic in some patients, pneumonitis commonly causes symptoms such as non-productive cough, shortness of breath, and low-grade fevers. Contrary to most other acute thoracic RT toxicities, radiation pneumonitis can develop into a potentially life-threatening situation, similar to an acute respiratory distress syndrome and is fatal in 1 to 2% of patients receiving thoracic chemoradiation [57]. To diagnose radiation pneumonitis, other differential diagnoses including tumor progression, infections, exacerbation of chronic obstructive pulmonary disease, or heart

failure, must first be ruled out. Thoracic imaging can be helpful and often reveals changes such as ground-glass opacities and nonspecific infiltrations in the irradiated areas of the lung. Management of radiation pneumonitis typically includes the administration of high-dose oral or intravenous corticosteroids, depending on severity, which are slowly tapered down over three to twelve weeks [58]. Pulmonary fibrosis is the late manifestation of radiation-induced lung injury. The molecular mechanisms are not yet fully understood. Patients commonly present with progressive chronic dyspnea months and years after radiation therapy. Parenchymal scarring can be seen in the irradiated portal on chest imaging, but these changes are also found in asymptomatic patients [59]. Treatment options are limited to symptom-relief, as fibrotic chances are very rarely reversable, and include inhalers and corticosteroids (inhaled or orally) [59]. Long after thoracic RT, the administration of systemic drugs including some chemotherapy and immunotherapy agents can lead to radiation recall pneumonitis.

Due to the proximity of the esophagus during irradiation of the lung or other mediastinal structures, thoracic radiation therapy can lead to acute or late esophageal toxicity. Following the first few weeks of radiotherapy, some patients will experience dysphagia, odynophagia and/or reflux-like symptoms. This may lead to dehydration and weight loss, which increases morbidity and can be a reason for treatment interruption or cessation [60]. Symptomatic management of acute esophagitis includes the application of topical anesthesia (i.e. oral viscous lidocaine), analgesics, acid-modifiers (histamin-2-blockers, proton-pump-inhibitors), and dietary changes [61]. Radiation esophagitis is usually self-limiting. In rare cases, esophagitis can manifest as a severe acute or late toxicity in form of bleeding, perforation, fistulation, stenosis, or stricture requiring dilation [61]. The esophageal mucosa has a relatively high tolerance for conventionally fractionated radiation. However, the tolerance level is lowered by the addition of concurrent chemotherapy to radiation therapy [62] and by hyperfractionation [63].

Radiation-induced skin changes, summarized as radiation dermatitis, are seen in thoracic radiation therapy as with radiation to any other part of the body. Up to 95% of patients treated with RT are estimated to develop radiation dermatitis of some degree during or after treatment [64]. The extent of the skin changes depends on a variety of factors including RT type, total dose, and fractionation, concurrent chemotherapy, as well as clinical factors such as comorbidities, skin integrity, and nutritional status [65]. Acute dermatological effects include redness as well as dry and moist desquamation. Late and chronic changes may present as pigmentation changes, photosensitivity, atrophy, fibrosis, and necrosis. Although most

dermatological manifestations resolve after the end of RT, the patient's quality of life can be markedly impacted by severe radiation dermatitis. There are still no wide-spread standards in radiation dermatitis management, but common treatment suggestions include topical moisturizers and steroids [65]. Attention must also be given to radiation recall dermatitis, an inflammatory response which can occur in a previously irradiated area of the skin after treatment with certain drugs including chemo- [66] and immunotherapy [67] agents.

Manifestations of radiation-induced heart disease include pericarditis, cardiomyopathy, heart failure, ischemic heart disease, valve regurgitation and stenosis as well arrythmia [68]. Reducing cardiac toxicity has long been of low priority in the treatment of advanced lung cancer. This was explained by the common understanding that cardiac toxicities take years or even decades to develop, as seen in many studies of lymphoma and breast cancer patients [69], and that the patient population of concern has a low life expectancy and a high probability of pre-existing cardiopulmonary comorbidities [70], making an accurate risk allocation of cardiac events challenging. More recent studies have demonstrated the urgent need for further research in this field, highlighting the potential acute cardiac toxicity. The RTOG (Radiation Therapy Oncology Group) 0617 study showed an association of the heart volume irradiated with 5 Gy or more (V5) and 30 Gy or more (V30) with worse overall survival [21]. Efforts are being made in achieving consistent dose reporting for the heart and identifying relevant heart substructures and resulting dose-volume constraints [68].

2.3.3. Immune Checkpoint Inhibition

The use of immune checkpoint inhibitors is associated with a unique toxicity profile known as immune-related adverse events (irAEs). Although the exact immunological mechanisms of these adverse events are not yet fully understood, the non-specific upregulation of immune pathways by ICIs is believed to play a central role in their development. A thorough clinical and laboratory workup before, during, and after ICI therapy as well as patient education is essential in diagnosing and managing irAEs. The American Society of Clinical Oncology (ASCO) issued a clinical practice guideline for the management of irAEs in patients treated with ICIs in 2018 [71]. In general, management of irAEs involves suspending ICI treatment (temporarily for most grade 2 to 3 irAEs, permanently for grade (3 to) 4 irAEs) and starting a glucocorticoid therapy [71]. There is conflicting evidence on whether the incidence of irAEs or the use of immunosuppressive agents for the treatment of irAEs have an influence on the

efficacy of the ICI but most data suggests no negative impact [72]. Patients with preexisting autoimmune diseases are commonly excluded from clinical studies with ICIs resulting in limited data on the safety of ICIs in this patient cohort. Although observational studies suggest that ICI are safe in most of these patients, the risk of specific irAEs like colitis, and an exacerbation of their existing autoimmune disorder seems to be higher than in the control population [73].

IrAEs include both systemic and organ-specific reactions. Fatigue, fever, chills, and infusion reactions can occur with all ICIs and are generally mild. The most common irAEs are dermatological, gastrointestinal, and endocrine. However, treatment with ICIs has been associated with a range of irAEs across all organ systems including renal (e.g., nephritis, acute kidney injury), neurological (e.g., headache, peripheral neuropathy, Guillain-Barré syndrome, myasthenia gravis, aseptic meningitis, encephalitis), cardiovascular (e.g., venous thromboembolism, myocarditis, vasculitis, arrhythmias), hematological (e.g., aplastic anemia, neutropenia, thrombocytopenia, autoimmune hemolytic anemia, cryoglobulinemia), ocular (e.g., episcleritis, conjunctivitis, uveitis), and rheumatological/musculoskeletal (e.g., myositis, inflammatory arthritis, sicca syndrome) [71,74].

Rash and/or pruritus are common findings in patients undergoing ICI treatment with early onset (two to five weeks after initiation of treatment) and most cases being mild and easily manageable. A maculopapular rash is typically seen with anti-PD-1/anti-PD-L1 antibodies but other dermatological manifestations of ICIs, including follicular, urticarial, or lichenoid dermatitis, bullous pemphigoid, vitiligo, alopecia, Sweet's syndrome, Stevens Johnson syndrome, as well as mucosal toxicities such as mucositis and gingivitis, have been reported [75]. Topical corticosteroids and oral antihistamines in cases of pruritus are often sufficient in managing low-grade dermatological irAEs and immunotherapy can normally be continued. Holding ICI treatment and initiating a systemic corticosteroid therapy should be considered in grade 2 and is standard for grade ≥ 3 dermatological irAEs [71]. Guidelines recommend a dermatological evaluation for atypical findings, grade ≥ 3 irAEs, manifestations that do not improve with initial therapy, and involvement of the oral mucosa [75].

Colitis and diarrhea occur frequently with CTLA-4 inhibitors and less often with PD-1 or PD-L1 inhibitors [76]. Diarrhea is defined as an increase of stool frequency above baseline, whereas colitis is associated with abdominal pain and clinical and/or radiographic/endoscopic signs of colonic inflammation. Symptoms typically begin to appear six weeks into treatment.

Differential diagnoses such as infections with Clostridioides difficile, cytomegalovirus or other bacterial or viral pathogens should be excluded before escalating treatment of the irAE. While symptomatic treatment including dietary changes and antidiarrheal medications are sufficient for grade 1 diarrhea/colitis, higher grade irAEs commonly require ICI suspension or discontinuation, oral or intravenous corticosteroids, and additional immunosuppression with infliximab (an anti-TNF antibody), if intravenous corticosteroids are not effective after three days [71]. Hepatic function should be monitored frequently during ICI treatment to detect signs of hepatitis, including elevated serum levels of aspartate aminotransferase, alanine aminotransferase, and bilirubin. While most patients will remain asymptomatic with laboratory changes, some can develop fever. Patients should be screened for viral- and drug-induced causes of hepatitis before and during ICI therapy.

Around 10% of patients treated with ICI develop clinically significant endocrine dysfunction, with hypothyroidism, hyperthyroidism, and hypophysitis being the most common endocrinopathies [77]. Other manifestations include thyroiditis, adrenal insufficiency, and type 1 diabetes mellitus. Continued clinical and laboratory (hormone) screening is important as non-specific symptoms such as fatigue and headache can also be a sign of thyroid or pituitary gland dysfunction. In addition to short-term high-dose glucocorticoids, which are warranted in more severe cases of endocrine dysfunctions, most patients will receive appropriate hormone replacement therapy (levothyroxine, hydrocortisone) which must typically be continued even after finishing ICI treatment [71]. In patients that respond well to hormone replacement and/or glucocorticoid therapy, ICI treatment can be continued as soon as acute symptoms have resolved and, when applicable, the necessary glucocorticoid therapy is below a certain dose threshold [71]. Insulin therapy should be initialized in patients with immunotherapy-induced type 1 diabetes. Due to the extensive destruction of pancreatic beta cells with ICI, a glucocorticoid or infliximab treatment will not be effective in these cases [78].

ICI-induced pneumonitis is a clinical diagnosis and presents as an inflammation of lung parenchyma with symptoms such as cough, fever, shortness of breath or thoracic pain. Although uncommon, it is a potentially severe or even fatal irAE and thus requires special attention. No characteristic patterns or features have been found in radiographical and pathological examinations. As with radiation pneumonitis, other explanations for the patients' symptoms such as infectious diseases, chronic pulmonary diseases and intrathoracic progression of the malignancy must be ruled out and bronchoscopy with bronchoalveolar lavage (BAL) may be considered to aid diagnosis. In grade 1 pneumonitis, ICI treatment should

be paused for two to four weeks and may be resumed if there is evidence of radiographic improvement or resolution. Grade ≥ 2 pneumonitis requires withholding ICI treatment and starting a corticosteroid therapy. Grade ≥ 3 cases can require additional immunosuppression with infliximab or mycophenolate mofetil and empirical antibiotics. ICIs should be permanently discontinued in patients that have grade ≥ 3 pneumonitis [71].

2.4. Radiation Protection, Mitigation, and Toxicity Modification

In addition to technological advances in radiation therapy, identifying pharmaceutics to decrease radiation toxicity has long been an active field of research. Different mechanisms of actions of these agents are possible. Radiation protectors would be given before RT, radiation mitigators during or immediately following RT, and radiation toxicity modifiers after the development of adverse events [58]. Amifostine, the first drug reducing RT toxicity to be approved by the FDA, can reduce xerostomia from chemo- and radiotherapy in head and neck cancers [79]. The compound has also been shown to reduce esophagitis and pneumonitis rates after thoracic RT in some studies [80]. However, a high heterogeneity and major methodical limitations in different trials currently limit the strength of the existing evidence [58]. In addition to the broad spectrum of adverse events associated with the drug, this has prevented amifostine from finding its way into routine clinical use [81]. Several other drugs have shown promising results in preclinical and small clinical studies. Angiotensin-converting enzyme inhibitors such as captopril and enalapril as well as angiotensin-II antagonists have demonstrated reductive effects on radiation-induced toxicity including pneumonitis [82]. Pentoxifylline, a phosphodiesterase inhibitor which downregulates the production of pro-inflammatory cytokines, is similarly being investigated for prophylactic use in thoracic RT [83].

2.4.1. Alpha-2-Macroglobulin

In addition to developing pharmaceutical compounds to prevent or treat radiation toxicity, research is focusing on identifying natural substances which potentially possess an intrinsic radioprotective effect. One of the substances under investigation is alpha-2-Macroglobulin (α2M). Human α2M is a glycoprotein and the largest non-immunoglobulin serum protein. It is known especially for its ability to inhibit virtually any proteinase and it binds and regulates

various proteins and cytokines in the human body [84]. In animal studies, an association between individual α2M levels and radiation effects has been described. A dose-dependent upregulation of α2M was found in plasma analyses of rats after receiving incremental doses of Cobalt-60 gamma rays [85]. After being exposed to 6.7 Gy of full body radiation, rats with endo- or exogenously increased levels of α2M showed significantly higher survival rates and regained baseline values such as body weight and lymphocyte count faster that the rats in the control group which had normal α2M levels [86,87]. Higher α2M levels led to normal or even enhanced proliferative ability of liver tissue following radiation compared to the rat control group [88]. In cell (human bone marrow mesenchymal stem cells) and rat models, pretreatment with α2M showed a protective effect against radiation injury [89], especially osteoradionecrosis of the jaw [90]. Key mechanisms which are thought to enable the radioprotective effect of α2M are the promotion of antioxidant enzyme expression, the inhibition of fibroblast activation (thus preventing fibrosis), the binding of pro-inflammatory cytokines, the restoration of homeostasis, and the enhancement of DNA and cell repair mechanisms [91].

3. Material and Methods

For both studies, data was collected retrospectively from patient electronic medical records at Memorial Sloan Kettering Cancer Center (MSKCC), New York and analyzed under institutional review board waivers.

3.1. Radiation Therapy + Immune Checkpoint Inhibitor Study

Materials and methods of this study have been previously published in part [95] and presented as an oral abstract at the 2016 Annual Meeting of the American Society for Radiation Oncology in Boston, Massachusetts, September 25 to 28, 2016 [96].

3.1.1. Patient Selection

To identify appropriate patients for this study, an institution-wide query via Dataline, MSKCC's patient data center, was commissioned. Search criteria were:

a) thoracic radiation therapy for any cancer and
b) treatment with any kind of immunotherapy.

After receiving results for this search, the patient list was further filtered by following criteria:

a) immunotherapy limited to following immune checkpoint inhibitors:
    i. PD-1 inhibitors
    ii. PD-L1 inhibitors
    iii. CTLA-4 inhibitors
b) radiation therapy to the lung or mediastinum
c) six months or less between any line of immunotherapy and any course of radiation therapy.

Subsequently, all data elements shown in the appendix were either retrieved from the query results, identified manually from the electronic medical records, or calculated using given data.

Patients received immunotherapy with either PD-1, PD-L1, CTLA-4 inhibitors or a combination of PD-1/PD-L1 and CTLA-4 inhibitors. Table 2 lists the ICIs that were included. RT was delivered in an accelerated scheme with palliative intention (30 Gy in ten fractions),

SBRT (18 to 50 Gy in three to five fractions) or conventionally fractionated RT (45 to 74 Gy in 20 to 47 fractions). The patient cohort included patients treated as part of prospective clinical trials as well as patients receiving treatment off trial.

Table 2. Immune checkpoint inhibitors included in study

PD-1	**PD-L1**	**CTLA-4**
Nivolumab	Durvalumab	Ipilimumab
Pembrolizumab	Atezolizumab	Tremelimumab

Following cut-off values were used for distinguishing the therapy timing: Concurrent therapy was defined as both therapies being receiving at the same time, regardless how long the overlapping amount of time was. Closely timed therapy was defined as less than four weeks interval between both therapies. Sequential therapy included patients in whom both therapies were given between four weeks and six months apart.

The primary endpoint of this study was the rate of adverse events following the combination of RT and immunotherapy. Only AEs that occurred after the start of the second therapy (either RT or immunotherapy) were included in the analysis. AEs were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, developed by the National Cancer Institute [92]. A list of all AE categories for which data was collected can be found in the appendix. The analysis focused on the most common and significant AEs, namely pneumonitis, other pulmonary AEs, esophagitis, fatigue, and dermatitis/rash/pruritus (collectively described as dermatological AEs). CTCAE v4.03 classifications for pneumonitis, esophagitis and radiation dermatitis can be found in Tables 3 to 5.

Table 3. CTCAE v4.03 classification for pneumonitis [92]

Grade 1	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
Grade 2	Symptomatic; medical intervention indicated; limiting instrumental ADLs
Grade 3	Severe symptoms; limiting selfcare ADLs; oxygen indicated
Grade 4	Life-threatening respiratory compromise; urgent intervention indicated (e.g. tracheotomy or intubation)
Grade 5	Death

Table 4. CTCAE v4.03 classification for esophagitis [92]

Grade 1	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
Grade 2	Symptomatic; altered eating/swallowing; oral supplements indicated
Grade 3	Severely altered eating/swallowing; tube feeding, total parenteral nutrition or hospitalization indicated
Grade 4	Life-threatening consequences; urgent operative intervention indicated
Grade 5	Death

Table 5. CTCAE v4.03 classification for radiation dermatitis [92]

Grade 1	Faint erythema or dry desquamation
Grade 2	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema
Grade 3	Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion
Grade 4	Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated
Grade 5	Death

In addition to the AE rates, data on the AE attribution was collected. Standard attribution classifications, namely “definitely”, “probably”, “possibly”, “unlikely”, and “unrelated”, were used throughout for the attribution to either RT or immunotherapy. Study records were screened for attributions for all grade 2 or higher AEs in the group of patients that were followed on clinical trial protocols. Retrospective assessment of attribution was implemented for patients that were treated outside of clinical trials. Factors that influenced the assessment included post-treatment time period till onset of AE, extent of toxicity in relation to RT treatment fields, and severity of toxicity in relation to RT doses.

3.1.2. Statistical Analysis

Patients and treatment characteristics as well as AE rates were analyzed using descriptive statistics. Univariate analyses were performed using Fisher's exact test and exact Wilcoxon rank-sum test for categorical and continuous variables, respectively. The aim was to examine the association between pneumonitis, esophagitis, and dermatological AE rates on one side and several clinical and treatment-related variables on the other side.

3.2. Alpha-2-Macroglobulin Study

Materials and methods of this study have been previously published in part [97] and presented as a poster at the 2016 Annual Meeting of the American Society for Radiation Oncology in Boston, Massachusetts, September 25 to 28, 2016 [98].

3.2.1. Patient Selection

The α2M study analyzed a patient cohort treated with thoracic RT at MSKCC between 2012 and 2016. Patients underwent treatment with either conventionally fractionated RT (3D-CRT or IMRT) or SBRT. Patients who had already received thoracic RT at an earlier time were excluded. Data was systematically collected on clinical, laboratory, treatment, and toxicity factors. Data collection took place during standard treatment and follow-up procedures at baseline (prior to begin of treatment) and at routine follow-up visits every three months for the first two years. Adverse event data consisted of CTCAE v4.03 graded radiation pneumonitis and esophagitis rates.

3.2.2. Alpha-2-Macroglobulin

One serum sample for α2M analysis was collected from each patient at baseline prior to fraction #1 of RT. Serum samples were taken during pre-treatment appointments 30 days or less prior to RT start, typically at the time of RT simulation. The mean time between measurement of α2M and the start of RT was 14 days with a standard deviation of six days. α2M testing was performed according to Clinical Laboratory Improvement Amendments criteria at Quest

Diagnostics Nichols Institute (San Juan Capistrano, California). Unit of measurement for α2M levels was mg/dL with the normal range being defined as 100 to 280 mg/dL.

3.2.3. Treatment Plans

Treatment plans for patients receiving RT before 2014 were retrieved from our in-house planning system [93]. Beginning in 2014, the Eclipse treatment planning system (Varian Medical Systems, Palo Alto, California) was used. Treatment plans were imported to the research platform CERR (Computational Environment for Radiological Research) [94] for the analysis of dosimetric data. The dosimetric variables used for the analysis were extracted from target structures. For esophagitis this was the complete esophagus, for pneumonitis 'lung minus gross tumor volume (GTV)' and heart. Preceding this step was the conversion of plan doses to equivalent dose in 2 Gy fractions with α/β ratio of ten for esophagus and three for lung minus GTV and heart. Dx values (minimum dose to the volume with the x% hottest dose in the organ of interest) were used in this analysis. An example of Dx and Vx extraction from a dose volume histogram (DVH) can be found in Figure 2. To account for the fact that radiation esophagitis tends to develop more acutely during RT than pneumonitis, an additional set of dosimetric variables other than the planned doses was extracted for esophagus. To this end, DVH bins were divided by the number of treatment days between start and end of RT (including weekends), resulting in fractional variables, identifiable by the prefix "f" (e.g., fmax dose).

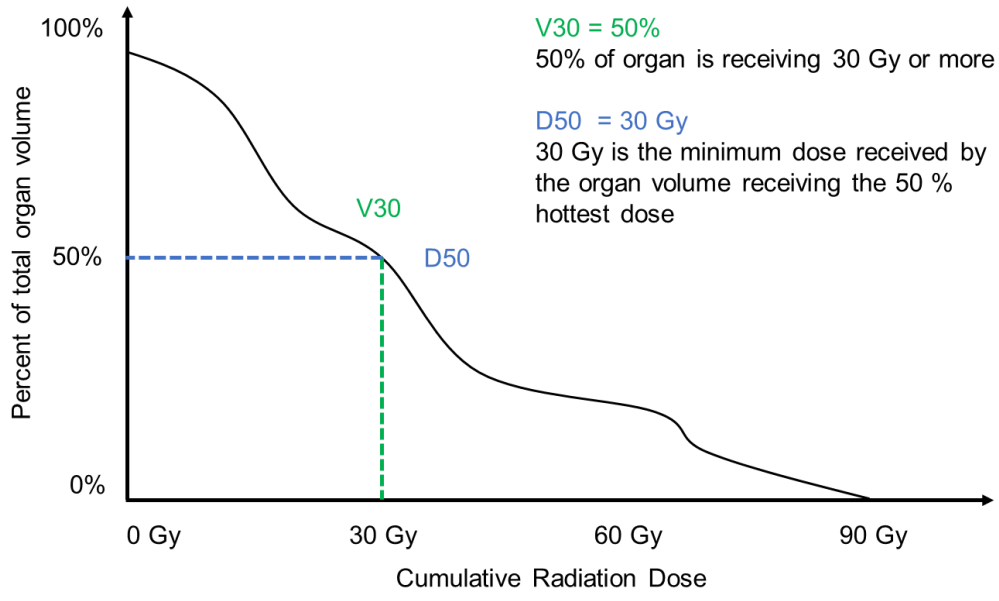


Figure 2. Example of a dose volume histogram with D50 and V30

3.2.4. Statistical Analysis

To investigate associations between adverse events (esophagitis and pneumonitis) and α2M levels, clinical, and dosimetric variables, both univariate and multivariate analyses were performed. For each endpoint, patients were categorized into two groups: non-toxicity (grade < 2) and clinically significant toxicity (grade ≥ 2).

Univariate analysis consisted of Wilcoxon rank-sum test and Spearman's correlation (Rs) test. The former was used to assess the difference in α2M expression between the two toxicity groups. For the Spearman's correlation test, CTCAE grades 0 to 5 were used instead of dichotomized values (grade < 2 and grade ≥ 2). This test was used to examine associations between AE rates, Dx values, mean dose, max dose, clinical variables, and α2M. Dx values were computed from x = 5% to x = 100% in intervals of 5%.

Using features with $p < 0.1$ that resulted from the univariate Spearman's correlation test, multivariate analysis was performed using the least absolute shrinkage and selection operator (LASSO) logistic regression. Pearson's correlation test was conducted among all dosimetric variables before the LASSO regression analysis to avoid variable instability due to high collinearity. For further LASSO modelling, only a relatively small group of variables was selected. This was achieved using a cutoff of Pearson's correlation coefficient > 0.75 to determine a single variable from a set of correlated variables that had the best correlation with the toxicity endpoint after hierarchical clustering.

The data set was split into two groups to enable rigorous verification of model validity. The splitting was performed separately for pneumonitis and esophagitis. The training data was composed of 2/3 and the validation data of 1/3 of the samples. Both data sets were balanced in terms of cancer subtypes and outcomes. The model building process was conducted using only the training data. Furthermore, to examine the stability of LASSO variable selection, a bootstrapped dataset generated from the training data was used. The resulting models were then tested with validation data. The performance was quantified by the area under the receiver operating characteristic curve (AUC). The AUC serves as a function of the true positive rate (sensitivity) and false positive rate (1 - specificity). The final results consisted of the average performance on the validation data for predictive models which were built using 1000 bootstrapped datasets.

R language (version 3.2.4), MATLAB (version 8.6.0; MathWorks. Natick, Massachusetts) and SPSS (version 24; IBM. Armonk, New York) were used for statistical analyses.

4. Results

4.1. Radiation Therapy + Immune Checkpoint Inhibitor Study

Results of this study have been previously published in part [95] and presented as an oral abstract at the 2016 Annual Meeting of the American Society for Radiation Oncology in Boston, Massachusetts, September 25 to 28, 2016 [96].

4.1.1. Patients

79 patients that met the inclusion criteria were identified for this study, treated between 2006 and 2015 at Memorial Sloan Kettering Cancer Center, New York. 48 patients (61%) were male and 31 female (39%). Median age was 60 years with a range of 21 to 93. Most patients were treated for lung cancer (n = 45; 57%) or melanoma (n = 15; 19%) with the rest being distributed as follows: renal cell cancer (n = 6), colorectal cancer (n = 4), squamous cell cancer (not lung, n = 4), sarcoma (n = 2), basal cell cancer (n = 1), breast cancer (n = 1), and rhabdomyosarcoma (n = 1). Three patients had a second type of cancer in addition to lung cancer (one melanoma, one colorectal cancer, one breast cancer). 44 patients (56%) received sequential, 15 (19%) closely timed and 20 (25%) concurrent treatment. First treatment was ICI for 43 patients (54%) and RT for 36 patients (46%). ICI consisted of PD-1 inhibitors in 48 (61%), PD-L1 inhibitors in 14 (18%), CTLA-4 inhibitors in 12 (15%) and combined inhibitor treatment (PD-1 or PD-L1 inhibitor and CTLA-4 inhibitor) in five patients (6%). 44 patients (57%) received ICI as part of a clinical trial. Most patients received an accelerated RT scheme in palliative intention (n = 46, 58%) while 18 (23%) received SBRT and 15 (19%) received conventionally fractionated RT. Median RT total dose in all patients was 30 Gy (range 18 to 78 Gy). The median follow-up time for all patients was 4.5 months (range 0.2 to 55.6 months) while median follow-up time for survivors was 5.9 months (range 2.4 to 55.6 months). Complete patient and treatment characteristics can be found in Table 6.

Table 6. Patient and treatment characteristics

Factor		n (total = 79)	%
Sex	Male	48	61
	Female	31	39
Median age (range), years			60 (21, 93)
Cancer type	Lung cancer	45	57
	Melanoma	15	19
	Other	19	24
Treatment timing	Concurrent	20	25
	Closely timed (within 4 weeks)	15	19
	Sequential (1 to 6 months)	44	56
First treatment	Radiation therapy	36	46
	Immunotherapy	43	54
Immunotherapy category	Anti-PD-1	48	61
	Anti-PD-L1	14	18
	Anti-CTLA-4	12	15
	Anti-PD-1/PD-L1 + anti-CTLA-4	5	6
Laterality of irradiated lesion	Right lung	40	51
	Left lung	27	34
	Mediastinum	12	15
Site irradiated lesion	Mediastinum	32	41
	Hilum	19	24
	Upper Lobe	14	18
	Lower Lobe	14	18
Fractionation	Accelerated	46	58
	SBRT	18	23
	Other	15	19
Median RT total dose (range), Gy			30 (18, 74)
Median follow-up (range), months			4.5 (0.2, 55.6)
Median follow-up for survivors (range), months			5.9 (2.4, 55.6)

4.1.2. Adverse Events

In total, 34 grade ≥ 2 pulmonary AEs were reported (Table 7). This included five patients (6.3%) with grade ≥ 2 pneumonitis (four grade 2 and one grade 4), pneumonia (n = 14; 17.7%), and upper respiratory infections (n = 5; 6.3%). Grade 4 pneumonitis occurred in a patient that received ICI three months after the completion of accelerated RT in palliative intention. Further

pulmonary AEs commonly described were dyspnea (n = 3), cough (n = 2), and pleural effusions (n = 3). Grade ≥ 2 esophagitis was experienced by six patients (7.6%), including five patients with grade 2 and one patient with grade 3. Other common grade ≥ 2 AEs included dermatitis/rash/pruritus (n = 8; 10.1%) and fatigue (n = 13; 16.5%).

Table 7. Adverse events grade (g) ≥ 2 after thoracic radiation therapy and immunotherapy

Adverse events (grade ≥ 2)		**G 2**	**G 3**	**G 4**	**G 5**	**G ≥ 2**	**G ≥ 2**
		n	n	n	n	n	%
Pneumonitis		4	0	1	0	5	6.3
Esophagitis		5	1	0	0	6	7.6
Dermatological AEs		8	0	0	0	8	10.1
Other Pulmonary AEs	Pneumonia	6	7	1	0	14	17.7
	Upper Respiratory Infection	5	0	0	0	5	6.3
	Dyspnea	3	0	0	0	3	3.8
	Cough	1	1	0	0	2	2.5
	Pleural effusion	0	3	0	0	3	3.8
	Pulmonary embolism	0	1	0	0	1	1.3
	Bronchopulmonary aspergillosis	0	1	0	0	1	1.3
Fatigue		8	5	0	0	13	16.5

AE attribution data was collected or assessed for following grade ≥ 2 AEs: pneumonitis, esophagitis, and dermatitis/rash/pruritus (dermatological AEs). Most cases of grade ≥ 2 pneumonitis were attributed to thoracic RT (four of five), as were most of the grade ≥ 2 esophagitis cases (five of six). Dermatitis was deemed to be most likely caused by ICI in the majority of cases (four of seven). Detailed attribution data can be found in Table 8. The median time to diagnosis for pneumonitis was 3.9 months after the start of the second treatment (range, 1.08 to 5.0 months), 0.46 months for esophagitis (range, 0.2 to 0.66 months), and 0.62 months for dermatological AEs (range, 0.03 to 5.82 months).

Table 8. Adverse event attribution to radiation therapy and immunotherapy

Case #	AE Grade	Attribution to RT	Attribution to immunotherapy
Pneumonitis			
P1	2	probably	possibly
P2	2	probably	possibly
P3	2	probably	unlikely
P4	2	probably	unlikely
P5	4	unlikely	probably
Esophagitis			
E1	2	probably	possibly
E2	2	probably	unrelated
E3	2	probably	unlikely
E4	2	definitely	unrelated
E5	2	unknown	unknown
E6	3	definitely	possibly
Dermatological AEs			
D1	2	unrelated	unrelated
D2	2	unrelated	probably
D3	2	unrelated	probably
D4	2	unrelated	probably
D5	2	unrelated	probably
D6	2	unlikely	unlikely
D7	2	probably	unlikely
D8	2	probably	unlikely

4.1.3. Univariate Analysis

When comparing treatment timing (concurrent, including closely timed, versus sequential treatment) and grade ≥ 2 pneumonitis, esophagitis, and dermatological AE rates, no statistically significant correlation was found (Tables 9 to 11). The immunotherapy drug category was the only significant variable for any grade ≥ 2 AE. It correlated with the rate of grade ≥ 2 esophagitis ($p = 0.04$) (Table 10). Six patients experienced grade ≥ 2 esophagitis, of these three (50% of cases) developed esophagitis after receiving anti-PD-L1 antibodies, two (33%) after

anti-CTLA-4 and one (17%) after anti-PD-1. None of the patients treated with a combination of anti-PD-1/anti-PD-L1 and anti-CTLA-4 antibodies developed esophagitis. There was no significant association found with sex, age, cancer type, first therapy (RT versus ICI), RT technique, RT laterality, or median RT dose. Given the absence of multiple significant variables on univariate analysis, further multivariate analysis could not be performed

Table 9. Univariate analysis of clinical and treatment characteristics and grade ≥ 2 pneumonitis

	Pneumonitis grade ≥ 2				
	No (n = 74)		**Yes (n = 5)**		***p* - value**
	n	%	n	%	
Sex					0.07
Female	27	36.5	4	80.0	
Male	47	63.5	1	20.0	
Median Age (range), years	60 (21, 93)		69 (47, 75)		0.28
Cancer type					1.00
Lung	42	56.8	3	60.0	
Melanoma	14	18.9	1	20.0	
Other	18	24.3	1	20.0	
Treatment timing					1.00
Concurrent	33	44.6	2	40.0	
Sequential	41	55.4	3	60.0	
First Therapy					0.37
RT	35	47.3	1	20.0	
ICI	39	52.7	4	80.0	
ICI category					1.00
Anti-PD-1	45	60.8	3	60.0	
Anti-PD-L1	13	17.6	1	20.0	
Anti-CTLA-4	11	14.9	1	20.0	
Anti-PD-1/PD-L1 + anti-CTLA-4	5	6.8	0	0	
RT laterality					0.45
Right lung	38	51.4	2	40.0	
Left lung	24	32.4	3	60.0	
Mediastinum	12	16.2	0	0	
RT technique					0.37
Accelerated	44	59.5	2	40.0	
SBRT	17	23.0	1	20.0	
Other	13	17.6	2	40.0	
Median RT total dose (range), cGy	3000 (1800,7400)		3000 (2400,6600)		0.62

Table 10. Univariate analysis of clinical and treatment characteristics and grade ≥ 2 esophagitis

	Esophagitis grade ≥ 2				
	No (n = 73)		**Yes (n = 6)**		***p* - value**
	n	%	n	%	
Sex					1.00
Female	29	39.7	2	33.3	
Male	44	60.3	4	66.7	
Median Age (range), years	61 (21, 93)		57 (32, 81)		0.74
Cancer type					0.07
Lung	44	60.3	1	16.7	
Melanoma	13	17.8	2	33.3	
Other	16	21.9	3	50.0	
Treatment timing					1.00
Concurrent	32	43.8	3	50.0	
Sequential	41	56.2	3	50.0	
First Therapy					0.21
RT	35	47.9	1	16.7	
ICI	38	52.1	5	83.3	
ICI category					0.04
Anti-PD-1	47	64.4	1	16.7	
Anti-PD-L1	11	15.1	3	50.0	
Anti-CTLA-4	10	13.7	2	33.3	
Anti-PD-1/PD-L1 + anti-CTLA-4	5	6.8	0	0	
RT laterality					1.00
Right lung	37	50.7	3	50.0	
Left lung	25	34.2	2	33.3	
Mediastinum	11	15.1	1	16.7	
RT technique					0.61
Accelerated	42	57.5	4	66.7	
SBRT	16	21.9	2	33.3	
Other	15	20.5	0	0	
Median RT total dose (range), cGy	3000 (1800, 7400)		3375 (2700, 4500)		0.78

Table 11. Univariate analysis of clinical and treatment characteristics and grade ≥ 2 dermatological AEs

	Dermatological AEs grade ≥ 2				
	No (n = 71)		**Yes (n = 8)**		***p* - value**
	n	%	n	%	
Sex					0.47
Female	29	40.8	2	25.0	
Male	42	59.2	6	75.0	
Median Age (range), years	60 (21, 93)		66 (44, 77)		0.63
Cancer type					0.12
Lung	40	56.3	5	62.5	
Melanoma	12	16.9	3	37.5	
Other	19	26.8	0	0	
Treatment timing					0.46
Concurrent	30	42.3	5	62.5	
Sequential	41	57.7	3	37.5	
First Therapy					0.72
RT	33	46.5	3	37.5	
ICI	38	53.5	5	62.5	
ICI category					0.17
Anti-PD-1	45	63.4	3	37.5	
Anti-PD-L1	12	16.9	2	25.0	
Anti-CTLA-4	9	12.7	3	37.5	
Anti-PD-1/PD-L1 + anti-CTLA-4	5	7.0	0	0	
RT laterality					1.00
Right lung	36	50.7	4	50.0	
Left lung	24	33.8	3	37.5	
Mediastinum	11	15.5	1	12.5	
RT technique					0.58
Accelerated	42	59.2	4	50.0	
SBRT	15	21.1	3	37.5	
Other	14	19.7	1	12.5	
Median RT total dose (range), cGy	3000 (1800, 7400)		3000 (2000, 5000)		0.43

When analyzing treatment timing in three distinct groups, the comparison of concurrent versus closely timed versus sequential timing similarly showed no significant correlation with any of the AE rates (Tables 12 to 14). *p* - values were 1.0, 0.85, and 0.27 for grade ≥ 2 pneumonitis, esophagitis, and dermatological AEs, respectively.

Table 12. Univariate analysis treatment timing and grade ≥ 2 pneumonitis

	Pneumonitis grade ≥ 2				
	No (n = 74)		**Yes (n = 5)**		***p* - value**
	n	%	n	%	
Therapy timing					1.00
Concurrent	19	25.7	1	20.0	
Closely timed (≤ 4 weeks interval)	14	18.9	1	20.0	
Sequential (> 4 weeks interval)	41	55.4	3	60.0	

Table 13. Univariate analysis treatment timing and grade ≥ 2 esophagitis

	Esophagitis grade ≥ 2				
	No (n = 73)		**Yes (n = 6)**		***p* - value**
	n	%	n	%	
Therapy timing					0.85
Concurrent	18	24.7	2	33.3	
Closely timed (≤ 4 weeks interval)	14	19.2	1	16.7	
Sequential (> 4 weeks interval)	41	56.2	3	50.0	

Table 14. Univariate analysis treatment timing and grade ≥ 2 dermatological AEs

	Dermatological AEs grade ≥ 2				
	No (n = 71)		**Yes (n = 8)**		***p* - value**
	n	%	n	%	
Therapy timing					0.27
Concurrent	16	22.5	4	50.0	
Closely timed (≤ 4 weeks interval)	14	19.7	1	12.5	
Sequential (> 4 weeks interval)	41	57.8	3	37.5	

4.2. Alpha-2-Macroglobulin Study

Results of this study have been previously published in part [97] and presented as a poster at the 2016 Annual Meeting of the American Society for Radiation Oncology in Boston, Massachusetts, September 25 to 28, 2016 [98].

4.2.1. Patients

A total of 258 patients were included in the analysis of this study. The median age was 69 years with 47% male und 53% female patients. Most patients were treated for NSCLC (78%). The majority of patients were former (70%) or current smokers (13%) with a median of 37 pack-years. Median follow-up time from the start of RT was 8.9 months. Further details on patient characteristics can be found in Table 15.

Table 15. Patient characteristics

Factor		**n (total = 258)**	**%**
Median Age (range), years		69 (25, 93)	
Sex	Male	122	47
	Female	136	53
Median Karnofsky performance status scale (range), %		90 (50, 100)	
Subgroups	NSCLC	202	78
	SCLC	17	7
	Thymoma	8	3
	Mesothelioma	25	10
	Lung metastases (other primary)	6	2
Smoking history	Never	47	18
	Former	179	69
	Current	32	12
Median Pack-Years of former/current smokers (range), years		37 (1, 204)	
Median Alpha-2-Macroglobulin (range), mg/dL		191 (94, 511)	
Chemotherapy timing	Concurrent	60	23
	Sequential	74	29
	No chemotherapy	124	48
Median RT total dose (range), Gy	Conventional RT	54 (27, 74)	
	SBRT	50 (30, 70)	
Median Follow-up time from start of RT (range), months		8.9 (0.2, 40.2)	

4.2.2. Toxicities

Grade 1 radiation esophagitis developed in 49 patients (19.0%), grade 2 in 53 (20.5%) patients and grade 3 in eight (3.1%) patients. There were no grade 4 or 5 esophagitis cases observed. Median time to development of esophagitis was 0.85 months after the start of RT (range 0.2 to 6.47 months). 28 patients (10.9%) experienced grade 1, 26 (10.1%) grade 2, nine (3.5%) grade 3 and one patient (0.4%) grade 4 radiation pneumonitis. We observed no grade 5 pneumonitis. Radiation pneumonitis developed at a median of 4.73 months after RT start (range 1.3 to 8.1 months).

The smoking status of the patients who experienced grade ≥ 2 esophagitis was "never" in eight (13.1%), "former" in 43 (70.5%), and "current" in ten (16.4%) patients. In patients that developed pneumonitis the smoking status distribution was nine (25%) "never", 24 (66.7%) "former", and three (8.3%) "current".

4.2.3. Univariate Analysis

4.2.3.1. Alpha-2-Macroglobulin

Spearman's correlation test showed a significant correlation between baseline α2M values and grade ≥ 2 esophagitis (Rs = -0.18 / p = 0.003). As shown in Table 16, the analysis with Wilcoxon rank-sum test demonstrated that patients with grade < 2 esophagitis displayed significantly higher baseline serum α2M levels than patients with grade ≥ 2 (p = 0.015). We found no statistically significant difference between baseline α2M levels and grade ≥ 2 pneumonitis (p = 0.837).

Table 16. Comparison of mean α2M serum levels between grade < 2 and ≥ 2 esophagitis and pneumonitis.
P-value was calculated using Wilcoxon rank-sum test.

Toxicity		**grade 0 or 1**	**grade ≥ 2**	***p* - value**
Esophagitis	n	197	61	0.015
	mean α2M (mg/dl)	208.9	190.4	
Pneumonitis	n	222	36	0.837
	mean α2M (mg/dl)	204.1	207.0	

There was a trend observed between smoking status and α2M levels. Compared to former (207.3 mg/dl) and never smokers (185.4 mg/dl), patients who were current smokers had higher mean levels (217.3 mg/dl). Similarly, former smokers had higher levels compared to patients that had never smoked. The α2M level showed a significant correlation with a grouped smoking status of "former" or "current" when compared to "never" (Rs = 0.13 / p = 0.04).

4.2.3.2. Clinical Factors

With univariate analysis, significant correlation with grade ≥ 2 esophagitis was seen for following clinical variables: age (Rs = -0.32 / p < 0.0001), total dose (Rs = 0.35 / p < 0.0001), dose per fraction (Rs = -0.57 / p < 0.0001), fraction number (Rs = 0.64 / p < 0.0001), treatment days (Rs = 0.60 / p < 0.0001), and chemotherapy use (Rs = 0.56 / p < 0.0001). The only variable showing significant correlation with grade ≥ 2 pneumonitis was sex (Rs = -0.32 / p = 0.037), with a higher risk for women.

4.2.3.3. Dosimetric Factors

Spearman's correlation test showed an association between all dosimetric variables in the esophagus and the development of grade ≥ 2 esophagitis with Rs > 0.6 (p < 0.0001) for all Dx, as seen in Figure 3A. Figure 3B shows the correlation of fractional doses (fDx) with grade ≥ 2 esophagitis. Here, the highest correlation was noted for fD40 (Rs = 0.58 / p < 0.0001). Lung and heart doses were assessed in patients with pneumonitis (Figure 3C).

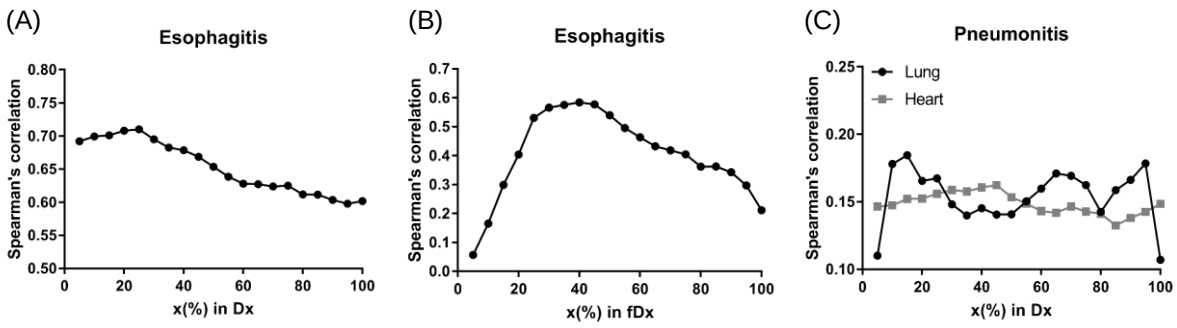


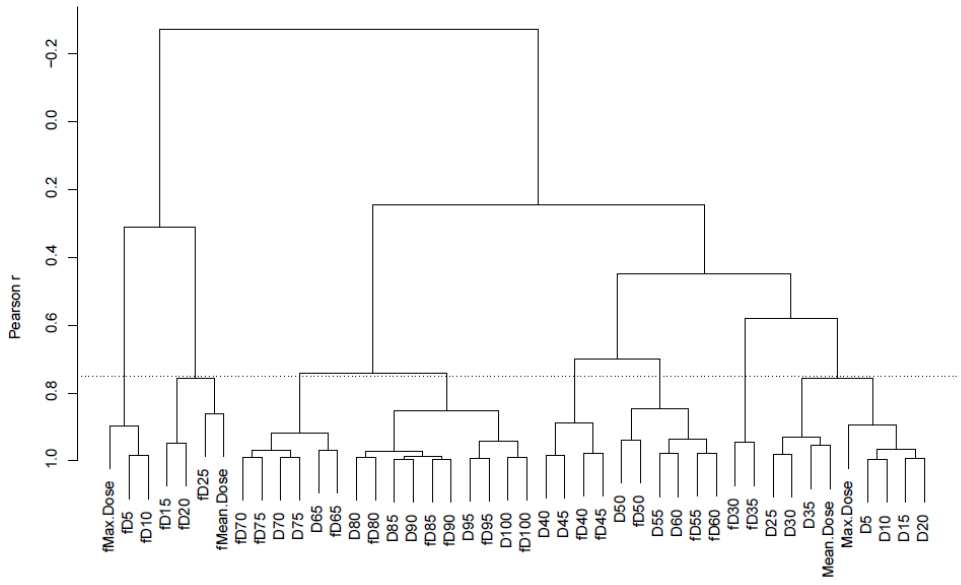
Figure 3. Spearman's correlation coefficients
Spearman's correlation coefficients between radiation-induced injuries (≥ grade 2) and Dx in esophagus for (A) esophagitis, fDx in esophagus for (B) esophagitis, and Dx in lung and heart for (C) pneumonitis.

D15 (Rs = 0.19 / p = 0.006) in lung and D45 (Rs = 0.16 / p = 0.016) in heart were found to be the highest correlated variables with grade ≥ 2 pneumonitis for each organ. Significant correlation with grade ≥ 2 pneumonitis was also noted for the maximum dose in heart (Rs = 0.14 / p = 0.043).

4.2.4. Multivariate Analysis and Validation Testing

To measure the similarity of the variables for multivariate analysis, hierarchical clustering together with Pearson's correlation test was performed on dosimetric variables of each organ using training data. As expected, there was a high correlation between many of the dosimetric variables, as seen in Figure 4. To select non-redundant variables for further testing, the variables were compared in clusters. The variable showing the highest correlation (Rs value) with the toxicity endpoint was selected among each cluster using a threshold of 0.75 in Pearson's correlation. For the LASSO logistic regression analysis, dosimetric variables selected through the clustering test and clinical variables with p < 0.1 in univariate analysis were used. For esophagitis, this included age, total dose, α2M, treatment days, SBRT (yes / no), and chemotherapy (yes / no) as well as D25, D40, D50, D65, D85, fD10, fD25, fD35 in esophagus (Figure 4A). For pneumonitis, the selected variables were treatment days, SBRT (yes / no), chemotherapy (yes / no), sex as well as D10, D15, D65, D95 in lung (Figure 4B) and D20, D45, max dose in heart (Figure 4C). Due to their high correlation with the variable of treatment days, the variables dose per fraction and number of fractions were excluded from the analysis.

(A)



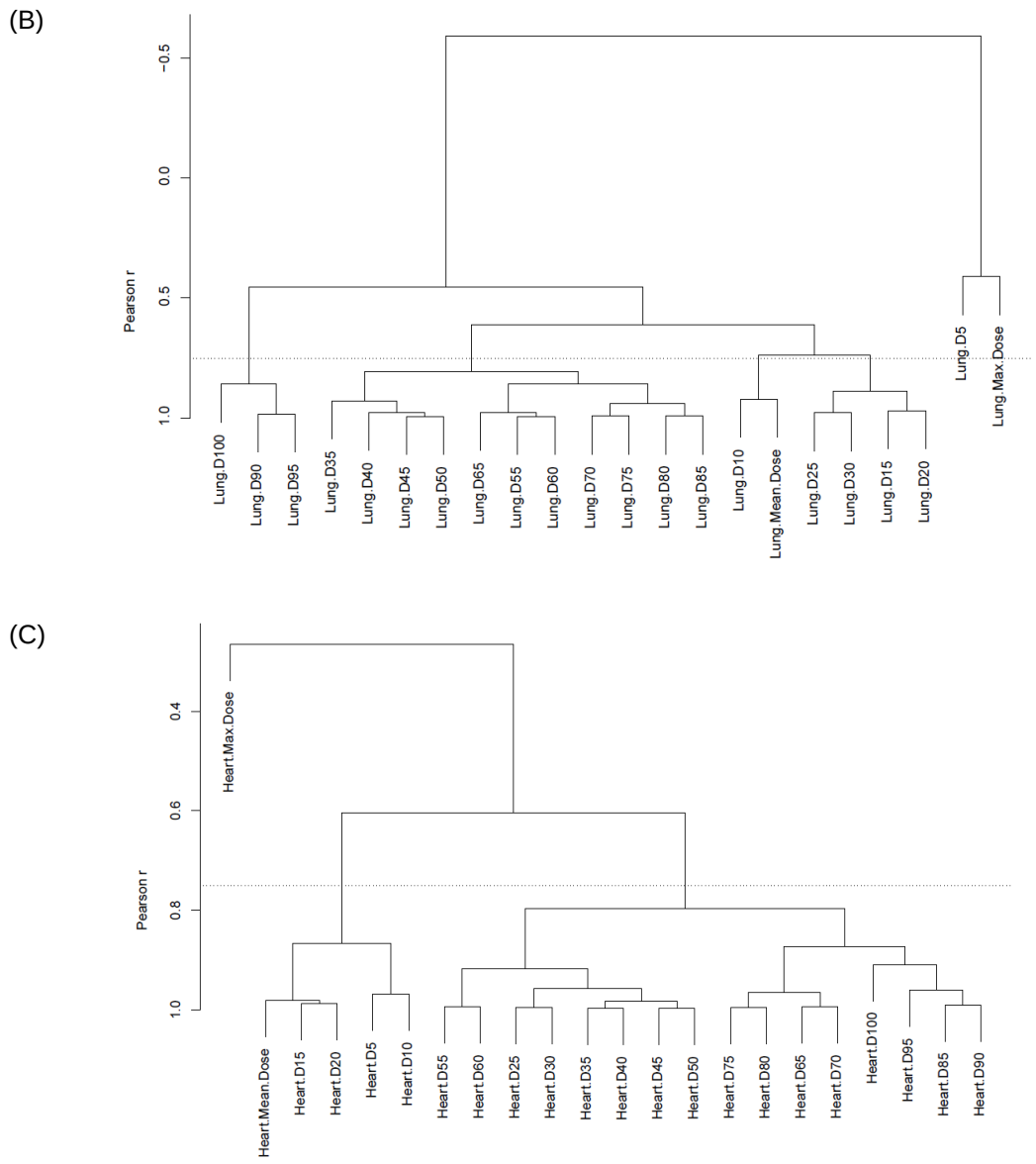


Figure 4. Pearson's correlation test
Using training data to remove redundant features with a threshold of 0.75: (A) dosimetric variables in esophagus for esophagitis, (B) in lung for pneumonitis, and (C) in heart for pneumonitis.

The training of the LASSO logistic regression models was achieved using 1000 bootstrapped datasets that were generated from training data. Testing was subsequently performed on the validation dataset. The average AUC after 1000 iterations, as seen in Figure 5, was 0.84 for esophagitis with a standard deviation (SD) of 0.03 and 0.78 for pneumonitis (SD = 0.06). For esophagitis, additional modeling was attained without the α2M variable. This resulted in the same average AUC of 0.84.

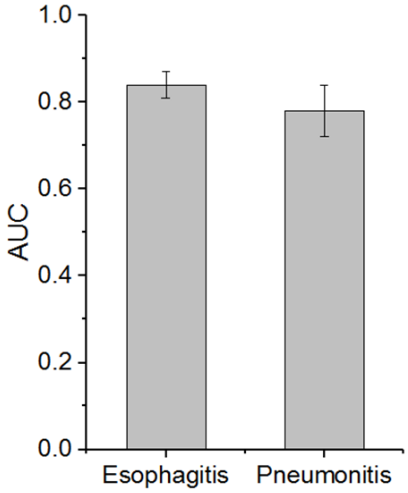


Figure 5. Average AUC after 1000 iterations of the LASSO logistic regression modeling on the validation data
The error bar indicates the standard deviation.

The frequency of each variable's inclusion during the model building process was evaluated to assess the respective feature's importance. In 1000 predictive models for esophagitis, the variables chemotherapy and treatment days were selected most frequently with 770 and 758 times, respectively (Figure 6A). α2M was the fifth most frequently included variable and was selected 610 times. In the same number of models for pneumonitis, D65 in lung and max dose in heart were most frequently selected variables, occurring 865 and 798 times, respectively (Figure 6B).

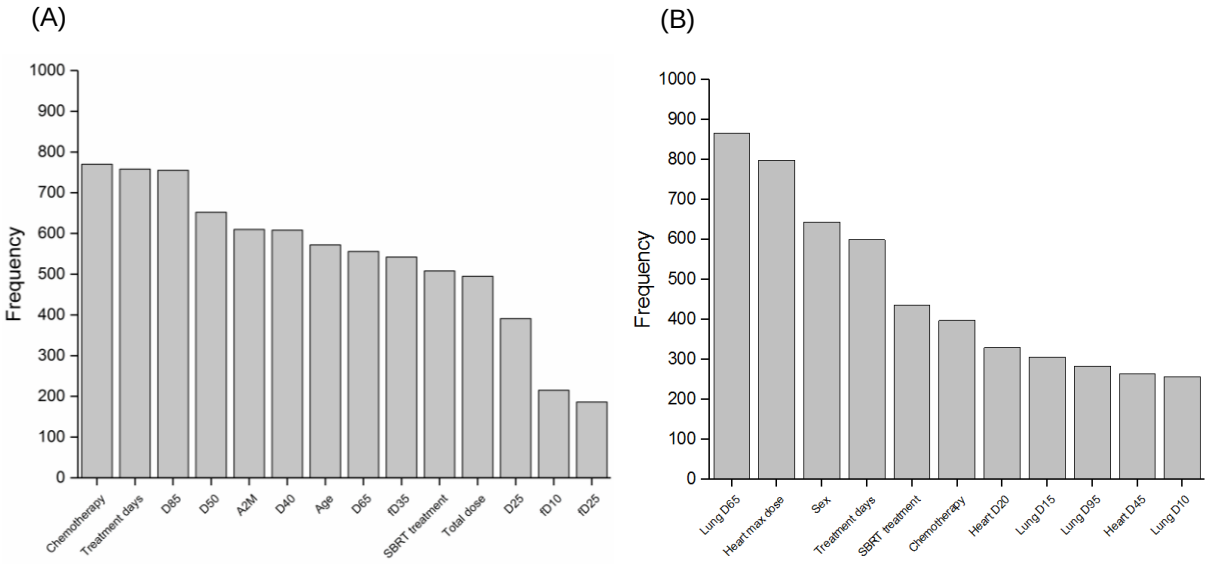


Figure 6. Frequency of occurrence of each feature used in 1000 predictive models
(A) esophagitis (B) pneumonitis

To further evaluate model robustness, we compared observed and predicted incidences of grade ≥ 2 esophagitis and pneumonitis. For this comparison, outcomes were predicted for patients using the validation data. In the next step, patients were sorted into six equal groups according to predicted risk of toxicity development. Group 1 included those patients with the lowest predicted risk, group 6 those with the highest risk. A high conformity of observed and predicted incidences was found for both esophagitis and pneumonitis (Figure 7), indicating highly robust predictive models.

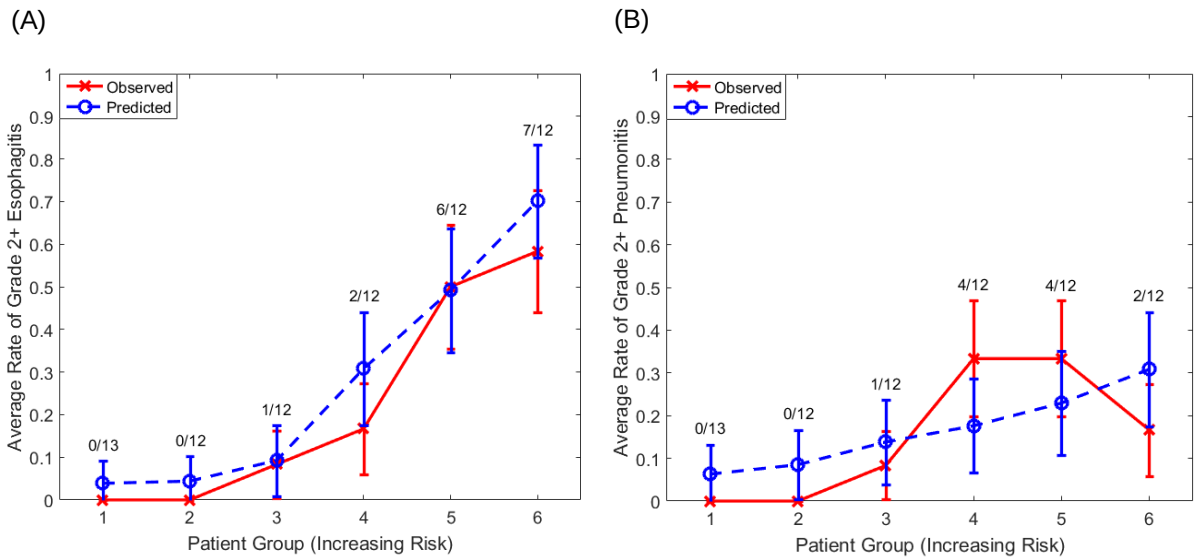


Figure 7. Comparison of observed and predicted incidence of grade ≥ 2 AEs in validation data
(A) esophagitis (B) pneumonitis
Numerator: number of events in each group. Denominator: number of samples in each group.

Table 17 and 18 show the final predictive models built using all training data. The final esophagitis model included the variables D25, D40, treatment days. The final pneumonitis model similarly included the variable treatment days, as well as D65 in lung and max dose in heart.

Table 17. Final predictive model for esophagitis
SD: standard deviation. CI: confidence interval

Variable	Coefficient	SD	Odds ratio	95% CI	
Esophagitis model					
D25	0.012	0.026	1.012	0.962	1.064
D40	0.036	0.022	1.037	0.993	1.083
Treatment days	0.048	0.026	1.049	0.997	1.105
Constant	**-3.880**	**0.725**	**0.021**	**0.005**	**0.086**

Table 18. Final predictive model for pneumonitis
SD: standard deviation. CI: confidence interval

Variable	Coefficient	SD	Odds ratio	95% CI	
Pneumonitis model					
D65 in lung	0.252	0.146	1.286	0.967	1.711
Max dose in heart	0.015	0.008	1.015	0.999	1.032
Treatment days	0.024	0.020	1.024	0.986	1.064
Constant	**-3.824**	**0.793**	**0.022**	**0.005**	**0.103**

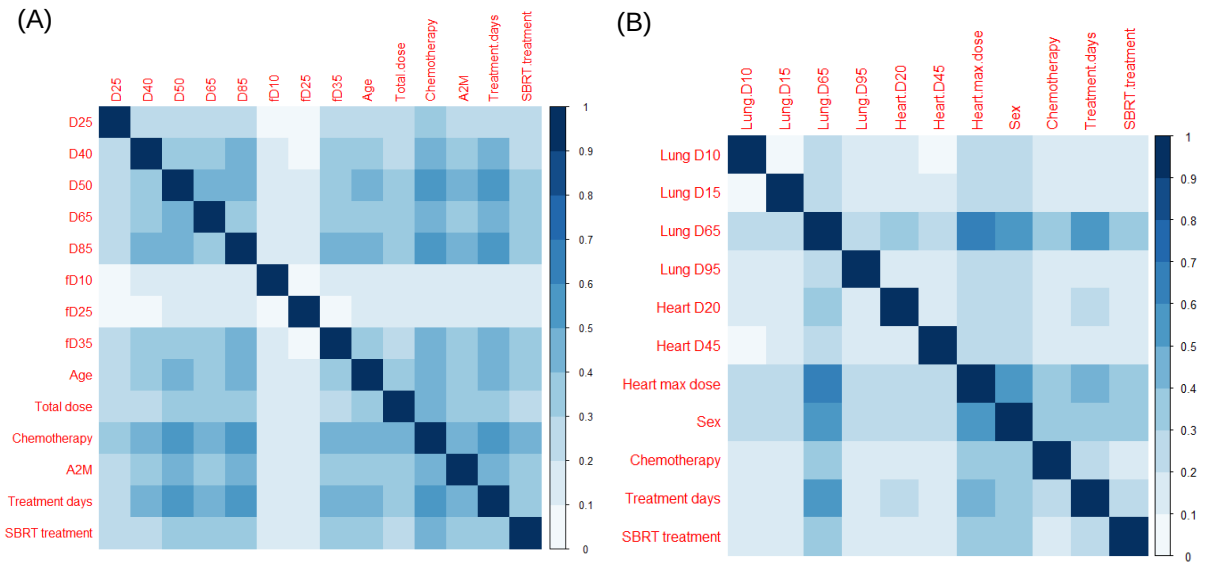


Figure 8. Frequency of occurrence of a pair of features (divided by 1000) used in 1000 predictive models
(A) esophagitis (B) pneumonitis

To further assess the interaction effects of features in the predictive models, the frequency of occurrence of separate pairs of features used in the LASSO logistic regression model was investigated, as shown in Figure 8. As also seen in Figure 6, the variable of sex is selected frequently in combination with D65 in lung and heart max dose in the pneumonitis models, even though it was not included in the final model.

5. Discussion

The aim of the studies presented in this thesis was to investigate aspects of two key issues in modern thoracic radiation therapy toxicity research. One of them being the toxicity of combined RT and ICI, the other being whether certain intrinsic factors or biomarkers can help predict radiation-induced toxicity. To our knowledge, at the time of their publication in 2018 [95] and 2020 [97], both studies were the largest to date of their kind, that is systematically comparing AEs in patients treated with thoracic RT and ICI in a concurrent versus sequential setting and investigating α2M as part of a predictive model for radiation pneumonitis and esophagitis, respectively.

Comparing AE rates for combined thoracic RT and ICI at the time our RT + ICI study was published was difficult given the limited amount of clinical data available up to that point. Accordingly, the toxicity rates were primarily discussed in the context of historic toxicity rates for thoracic RT and ICI monotherapy. Fortunately, the past few years have seen a stark increase in the availability of toxicity data, both for ICI alone as well as RT plus ICI in the setting of thoracic malignancies, adding a new dimension to the discussion.

Our RT + ICI study showed a grade ≥ 2 pneumonitis rate of 6.3%, a grade ≥ 3 rate of 1.3% and no grade 5 cases. Definitive concurrent thoracic chemoradiation typically leads to symptomatic (grade ≥ 2) radiation pneumonitis rates between 10% and 40% [57,99,100], while non-curative RT doses and advanced radiation techniques like IMRT, optimizing the sparing of healthy lung tissue, are associated with lower incidences [101,102]. Although not significant in univariate analysis, our RT + ICI study included a majority of patients that were treated with an accelerated thoracic RT scheme in palliative intention, as compared to SBRT and other RT techniques (58% versus 23% and 19%, respectively), which may contribute to the lower observed pneumonitis incidence. Immunotherapy-related pneumonitis is less common but can still be a potentially fatal adverse effect. In clinical trials including solid tumors, overall pneumonitis rates with monotherapy or combination ICI (anti-PD-1/PD-L1 plus anti-CTLA-4 antibodies) between 0 and 12% and grade ≥ 3 pneumonitis rates of 0 to 4.3% were observed [103–106]. Patients with NSCLC as well as patients receiving combination ICI appear to be at a higher risk of developing pneumonitis compared to other malignancies and ICI monotherapy. Large retrospective studies presented in the last few years have highlighted a higher incidence of symptomatic immune-related pneumonitis found ranging between 4% and 19% [103,107],

possibly due to increased use of ICI and the correlating heightening of clinician awareness and pharmacovigilance.

Often, the most significant acute toxicity seen during thoracic radiation is radiation esophagitis. Our analysis resulted in grade ≥ 2 esophagitis rates of 7.6% with one case of grade 3 esophagitis (1.3%) and no higher-grade cases. This is comparable with reported higher-grade esophagitis / dysphagia rates from concurrent chemo-RT (22 to 44%) [55,100,108] as well as palliative RT (0 to 54%) [109–112]. We did not see a significant change in esophagitis rates when comparing treatment timing. The only significant variable on unilateral analysis was the type of immune checkpoint inhibitor, with anti-PD-L1 inhibitor treatment displaying the highest esophagitis rate compared to PD-1 and CTLA-4. As immune checkpoint inhibitors so far have not exhibited adverse events in the upper gastrointestinal tract (namely dysphagia / esophagitis) but rather AEs such as colitis and diarrhea, there is little to no data regarding the impact on ICI therapy on the development of esophagitis in patients that may receive thoracic radiation in a similar time frame. This was reflected in our finding that most esophagitis cases in our study were attributed to thoracic RT (five of six cases) rather that to immunotherapy.

Dermatological toxicity occurs very frequently, both with (thoracic) RT as well as ICI, but rarely manifests in a severance which is dose-limiting or requires a treatment break or stop. There is evidence that dermatological complications appear at a slightly higher rate with ipilimumab compared to nivolumab or pembrolizumab (all grade: 50% vs. 30 to 40%) while grade ≥ 3 cases are seen in less than 5% of patients [76,113]. RT (with or without chemotherapy) is associated with grade ≥ 2 radiation dermatitis rates of 1 to 7% [41,114]. We saw a grade ≥ 2 dermatological AE rate of 10.1% in our study with no grade 3 or higher cases which is in line with existing literature on thoracic RT and ICI alone. No correlation with type of ICI was seen in our analysis which may be due to the small overall number of AEs detected.

Finally, results from the RT + ICI study seem to be comparable with results from similar investigations. Several retrospective studies reported pneumonitis or dermatitis rates in patients treated both with thoracic RT and ICI in a time period of up to one year [115–120]. Most of them show no significant increase, especially of grade ≥ 3 pneumonitis, compared to RT or ICI alone. But, as with RT alone, definitive radiation doses, especially when delivered as SBRT may be associated with slightly higher pneumonitis rates in combination with ICI compared to palliative RT plus ICI [118,119]. In a secondary analysis of the phase I trial KEYNOTE-001 (pembrolizumab in patients with advanced/metastatic solid tumors) Shaverdian et al. found no

significant increase of all grade or grade ≥ 3 pneumonitis in patients who had received previous thoracic RT, with the median time between RT and the start of ICI being 11.5 months [39].

As combined ICI and RT treatment attracts increasing attention, the issue of treatment timing becomes essential. In our analysis, we saw no significant differences in AE rates of pneumonitis, esophagitis, or dermatitis when comparing concurrent, closely timed or sequential treatment settings. Similarly, the sequence of treatment (RT or ICI first) did not result in differences in AE rates. Overall, our results seem to have been confirmed by promising data provided by several prospective clinical trials implementing concurrent thoracic RT / chemo-RT and ICI in the past two years. A post-hoc analysis of the phase III PACIFIC trial (durvalumab versus placebo as consolidation therapy after concurrent chemoradiation in 709 patients with stage III NSCLC) by Faivre-Finn et al. showed that any grade pneumonitis was proportionally more common after durvalumab versus placebo, but grade ≥ 3 pneumonitis rates were low overall and comparable between both groups. When stratified by time from RT end to randomization, grade 3 and 5 pneumonitis rates were also similar (< 14 days: 4.2%, 0% [durvalumab] vs. 1.7%, 3.3% [placebo]; ≥ 14 days: 3.4%, 1.4% [durvalumab] vs. 2.9%, 1.7% [placebo] respectively) [41]. However, as the authors note in their discussion, these results should be interpreted with care as it is possible that patients who were randomized quickly following concurrent chemo-RT recovered faster from the preceding treatment due to smaller tumor volume and/or lower RT dose to organs at risk. As there are no guidelines or concluding evidence concerning treatment timing, the range of concurrent and sequential treatment schemes is large. The only grade ≥ 3 pneumonitis case (grade 4) in our analysis occurred in a patient who received ICI 3.5 months after completion of accelerated RT in palliative intention. In several phase I and II trials of “true” concurrent (chemo-)RT and ICI, each including between 20 and 80 patients, grade ≥ 3 pneumonitis rates of 0 to 10% were demonstrated [121–124]. 0% pneumonitis rates were only seen in cohorts which did not receive concurrent but rather sequential chemo-RT while ICI was given concurrently with RT [121,124]. Timing of ICI after (SB)RT in different clinical trials included one day [125], one week [126], and four to eight weeks [127]. The corresponding reported grade 3 or higher pneumonitis rates were 17% (n = 1, grade 3) with nivolumab following lung SBRT (six patients), 0% (n = 0) with pembrolizumab following lung SBRT in a study of 20 patients, and 6.5% (n = 6 including four grade 3, one grade 4, and one grade 5) with pembrolizumab following concurrent chemo-RT (93 patients), respectively.

Most studies focus their safety analysis on the incidence of pneumonitis, an AE harboring a high risk of limiting treatment dosage and potentially leading to termination of treatment or even death. Meanwhile, limited information is available on dermatological AE and esophagitis rates in ICI + RT in concurrent versus sequential settings. Retrospective analyses demonstrated all grade dermatological AE rates of 20 to 27% with RT plus ICI, with no grade 3 or higher AEs. Regarding treatment timing, three trials of concurrent (chemo-)RT and ICI provided symptomatic dermatological AE rates of 0 to 21% with 5 to 10% grade 3 in one study as well as symptomatic esophagitis rates of 5 to 38% with no grade 3 or higher AEs [121–123]. The PEMBRO-RT study by Thelen et al. reported 29% all-grade and 6% grade ≥ 3 dermatological AEs in the experimental (pembrolizumab seven days after SBRT) versus 17% and 0% in the control arm (pembrolizumab alone) in recurrent metastatic NSCLC [126]. This supports our findings that combination RT and ICI does not lead to significantly higher grade ≥ 3 AEs than RT or ICI alone and there seems to be no significant difference in any or high-grade esophagitis or dermatological AE rates when comparing concurrent and sequential treatment settings.

As highlighted above, lung and esophageal injury, namely pneumonitis and esophagitis, remain two of the most challenging dose-limiting adverse events in radiotherapy of thoracic malignancies. Predictive modeling provides a multifactorial approach to identifying determinants in the development of toxicity. Classically, dosimetric parameters are the main components of these predictive models, but biological and genetic factors are becoming increasingly important. We integrated the proposed intrinsic radioprotectant alpha-2-Macroglobulin into our predictive analysis in addition to dose-volume metrics, treatment-related variables like chemotherapy and patient-individual factors.

Tissue damage from radiation stems from the impact of reactive oxygen species (ROS) on DNA as well as the cellular damage which stimulates the expression and secretion of proinflammatory, profibrotic and chemotactic cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β [128]. Alpha-2-macroglobulin has been identified as a protein with possible radioprotective qualities in preclinical studies [86–90,129] as well as a predictive factor correlating highly with the occurrence of radiation pneumonitis in proteomics analyses [130,131]. Several potential mechanisms for this effect have been described, some of them being the binding of proinflammatory and profibrotic cytokines by α2M as well as inducing the upregulation of antioxidant enzymes like manganese superoxide dismutase (MnSOD) [91,132]. Our analysis

showed a correlation of baseline human serum α2M levels and the patients' smoking status. Former and current smokers displayed higher mean α2M levels compared to never smokers. Similar findings have been previously reported [133] but other factors such as age and gender may also influence α2M levels independently of smoking status [134–138]. For example, studies show that females display up to 20% higher levels of α2M than same-aged males and α2M levels decrease with higher age [139]. There is evidence that active and former smoking is associated with lower incidences of radiation pneumonitis in patients receiving thoracic radiation [140–145]. An explanation of this phenomenon could lie in the effects that smoking triggers in the immunological microenvironment of the lung, leading both to immunosuppression as well as aggravated autoimmunity. This inflammatory stimulus could, in turn, also be the reason for the observed higher levels of α2M in individuals exposed to tobacco smoke.

Of the 258 patients included in the α2M study, 61 (23.6%) developed symptomatic (grade ≥ 2) radiation esophagitis. On univariate analysis, we saw a correlation between lower natural baseline levels of serum α2M and an increased incidence of radiation esophagitis in patients receiving radiation therapy for thoracic malignancies. However, our LASSO logistic regression models did not yield different results when performed with or without α2M. We hypothesize that this may be due to more significant dosimetric and clinical variables. When examining the frequency of occurrence of each feature in the model building process, it is worth noting that α2M was selected 610 times in 1000 different models, being the 5th most frequent feature. To our knowledge there is no other literature to date analyzing a connection between serum α2M and the development of radiation esophagitis. Our predictive models for radiation esophagitis included chemotherapy, treatment days, several dosimetric factors as well as age. The final model included D25, D40 and treatment days. This is in line with available data which has repeatedly shown significant correlation of radiation esophagitis rates with mean and max esophageal dose [62,146–149], the percentage volume of the esophagus receiving at least a certain dose (commonly V40 – V60) [149–153], as well as chemo-RT, especially when given concurrently [62,148,150,154,155]. Conflicting and thus inconclusive findings are available regarding the influence of age [150,156,157] and treatment duration / time [156–158]. The search for predictive biomarkers, including genetic parameters, cytokines, and proteins, is gaining increased attention as potential predictors in the development of radiation toxicity. Baseline IL-8 [159], acute phase responses (high platelet count, low hemoglobin level) [160], and different single nucleotide polymorphisms (SNPs) [158,161,162] have been found to be

associated with radiation esophagitis. However, pretreatment levels of a panel of cytokines did not increase predictive power for grade 3 radiation esophagitis in a different study [163].

14% of patients included in our analysis developed symptomatic radiation pneumonitis. Although previously described by Oh et al. [130] and Lee et al. [131], we did not see a correlation of α2M levels and radiation pneumonitis in our study. While our patient cohort was significantly larger than those two studies, both the pneumonitis rate as well as the rate of current smokers was lower in comparison, which may have an impact on the correlative analysis. As for esophagitis, the search for biomarkers predicting radiation pneumonitis is ongoing but has yet to produce conclusive results. An association has been shown for several SNPs [164,165] as well as cytokines such as TGF-β_1 (in blood or BAL) [166,167], IL-1α [168], IL-6 (in blood or BAL) [166,168,169], and the neutrophil-lymphocyte ratio [170], among others. D65 to the lung and max dose to the heart were most frequently selected in the predictive models and were part of the final model as well as the variable of treatment days. This validates previous reports on the importance of heart dose during thoracic radiation in predicting radiation pneumonitis [131,171,172]. However, heart irradiation was not found to be predictive of radiation pneumonitis in a large retrospective study including 629 NSCLC patients [173]. Our univariate analysis showed a higher risk for woman and the variable of sex was the 3rd most frequent feature chosen in 1000 models. This has been described before [174,175], but most studies do not find this correlation [142,144,176–178]. The most frequent dose parameters reported to be associated with radiation pneumonitis are V20 and mean lung dose (MLD), both with conventional RT [142,179,180] as well as SBRT [181,182]. As dosimetric variables are typically very collinear, there is yet no consensus on which exact dosimetric factors can reliably predict radiation toxicity, especially pneumonitis. Dose-volume constraints recommendations for conventional RT (MLD less than 20 to 23 Gy and V20 less than 25 to 35%) were described in the context of the *Quantitative Analysis of Normal Tissue Effects in the Clinic* (QUANTEC) project [183] and further investigated in the *Systematic Analysis of Toxicity after Radical Irradiation: Pneumonitis and Esophagitis* (STRIPE) project [57]. The American Association of Physicists in Medicine's Working Group on Biological Effects of Stereotactic Body Radiotherapy recently published similar recommendations for thoracic SBRT (MLD less than 6 to 8 Gy and V20 less than 10 to 15%) [184].

Both of the presented studies are subject to the obvious limitations of retrospective analyses. Although AEs were prospectively graded in both studies according to our clinical standard and α2M levels were collected before the start of RT, there remains a significant heterogeneity of both study populations, warranting caution while interpreting the results. While the inclusion requirements were clearly specified, our data still includes a range of tumor types and histology, immuno- and chemotherapy types, RT dose and fractionation schemes as well as intervals between therapies. Both studies may have been limited in their statistical power by a relatively low overall incidence of AEs. At our institution and specifically for these two studies, AEs are graded according to CTCAE, with grade two or higher AEs being defined as symptomatic AEs. When discussing different studies regarding toxicity rates, a direct comparison is often impeded by the use of various grading schemes and grade cutoff values for analysis (i.e. including grade ≥ 2 or ≥ 3 AEs). The median follow-up period of 5.9 months in the RT + ICI study was relatively short due to the fact that many patients were treated with immunotherapy after multiple prior lines of therapy and in advanced stages of their disease. Nevertheless, we believe the follow-up time was acceptable of the specific endpoints of the study as it is long enough to capture acute AEs and the majority of radiation pneumonitis cases, which typically occur within one to six months after thoracic RT. In the α2M study, attention must be given to the fact that there are several known (and probably many more unknown) factors influencing intrinsic α2M levels. This increases the range of α2M values in the analyzed cohort and makes an attribution of potential radioprotective effects challenging because α2M-influencing factors like age and gender have also been individually associated with changes in toxicity rates. Furthermore, the only time α2M levels were measured in our study was in a 30-day period before the start of RT. Additional α2M measurements shortly before and during RT would be needed to expand our understanding of RTs influence on α2M levels and α2M's influence on the development of AEs.

Radiation therapy is an essential element in the treatment of thoracic malignancies and metastases. Due to improving technique and expansion of use, its importance will most likely continue to rise. Bearing this in mind, understanding, predicting, and preventing serious and treatment-limiting toxicity is crucial. Our studies provided insight into two current research topics in this field. The RT + ICI study was one of the first at its time of publication delivering evidence that the combined treatment is not associated with a significant increase in toxicity, especially radiation pneumonitis, esophagitis, and dermatitis, when delivered concurrently or

sequentially in a specified time period. Although the existing data does not allow a definitive conclusion regarding the safest and most efficacious timing of ICI and RT, this is nevertheless encouraging from a patient and practitioner perspective. Results from ongoing clinical studies of RT and ICI for several malignancies will hopefully provide a more conclusive foundation for this treatment approach in the future. Aside from treatment-related factors, development of toxicity is also influenced by clinical and intrinsic factors. In our α2M study, we could only register a univariate correlation of α2M levels and radiation esophagitis. However, we could validate existing predictive models and factors found to be predictive of radiation pneumonitis and esophagitis. In all treatment settings, special attention should be given to dose volume restrictions during treatment planning, as this is vital in reducing potential toxicity, both for RT alone as well as in combination with systemic treatment options.

Zusammenfassung

Strahlentherapie ist ein wesentlicher Bestandteil der Behandlungsstrategie von malignen Erkrankungen im Thorax. Die Entwicklung von Immuncheckpoint-Inhibitoren (ICI) bietet neue Möglichkeiten der Kombination mit Strahlentherapie, jedoch besteht noch viel Unklarheit bezüglich der Sicherheit dieses Therapieansatzes. Weitere Forschungsschwerpunkte im Bereich der Toxizität von thorakaler Strahlentherapie sind die Identifizierung von Radioprotektoren und prädiktiven Faktoren für unerwünschte Ereignisse.

In der Strahlentherapie plus ICI Studie wurde das Auftreten der häufigsten unerwünschten Ereignisse bei Patienten, welche innerhalb eines 6-Monats-Zeitraums sowohl thorakale Bestrahlung als auch eine ICI-Therapie erhielten, ausgewertet. Die alpha-2-Macroglobulin (α2M) Studie analysierte Patienten, welche sich einer thorakalen Strahlentherapie unterzogen hatten und für die Serumwerte von α2M, einem Glykoprotein, welches in präklinischen Studien eine radioprotektive Wirkung gezeigt hatte, vor Beginn der Therapie vorhanden waren. Um den Einfluss auf die Entstehung von unerwünschten Ereignissen zu untersuchen, wurden für Pneumonitis und Ösophagitis prädiktive Modelle errechnet, welche sowohl die α2M-Werte als auch klinische und dosimetrische Variablen enthielten.

In der ersten Studie sahen wir Grad ≥ 2 Pneumonitis, Ösophagitis und Dermatitis in jeweils 6,3, 7,6 und 10,1% der 79 Patienten. Der Zeitraum zwischen beiden Therapien (gleichzeitig, ≤ vier Wochen, > vier Wochen) hatte keinen Einfluss auf die Inzidenz der unerwünschten Ereignisse. In der α2M Studie zeigten sich Grad ≥ 2 Pneumonitis und Ösophagitis in jeweils 14 und 23,6% der 258 Patienten. Der mediane α2M-Wert war bei Rauchern und ehemaligen Rauchern (217,3 bzw. 207,3 mg/dl) signifikant höher als in Patienten, die noch nie geraucht hatten (185,4 mg/dl) und es zeigte sich eine univariate Korrelation von α2M-Werten mit dem Auftreten von Ösophagitis. Die prädiktiven Modelle bestanden für Pneumonitis aus den Variablen D65 (Lunge), Maximaldosis (Herz) und der Anzahl der Therapietage und für Ösophagitis aus D25, D40 (beides Ösophagus) und der Anzahl der Therapietage.

Die vorliegenden Studien zeigten keine signifikant höheren Raten von unerwünschten Ereignissen nach einer Behandlung mit thorakaler Strahlentherapie und ICI verglichen zu einer jeweiligen Monotherapie und auch der zeitliche Abstand zwischen den Therapien hatte hierauf keinen Einfluss. Prätherapeutische α2M-Werte waren nicht mehr in unseren endgültigen prädiktiven Modellen vorhanden, jedoch konnten wir den Zusammenhang zwischen α2M und dem Raucherstatus und sowie den Einfluss von verschiedenen klinischen und dosimetrischen Variablen auf das Entstehen von Pneumonitis und Ösophagitis bestätigen.

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List of Figures

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Acknowledgements

The acknowledgements are not in included in this version for data privacy protection.

Eidesstattliche Erklärung

Ich erkläre, dass ich die in der Medizinischen Fakultät der Otto-von-Guericke-Universität zur Promotion eingereichte Dissertation mit dem Titel

Understanding Toxicity of Thoracic Radiation Therapy – The Influence of Immune Checkpoint Inhibitors and Alpha-2-Macroglobulin

in der Universitätsklinik für Strahlentherapie der Otto-von-Guericke-Universität Magdeburg

mit Unterstützung durch Prof. Dr. Günther Gademann

ohne sonstige Hilfe durchgeführt und bei der Abfassung der Dissertation keine anderen als die dort aufgeführten Hilfsmittel benutzt habe.

Bei der Abfassung der Dissertation sind Rechte Dritter nicht verletzt worden.

Ich habe diese Dissertation bisher an keiner in- oder ausländischen Hochschule zur Promotion eingereicht. Ich übertrage der Medizinischen Fakultät das Recht, weitere Kopien meiner Dissertation herzustellen und zu vertreiben.

Magdeburg, den 26.11.2021

Donata von Reibnitz

Curriculum Vitae

The curriculum vitae is not in included in this version for data privacy protection.

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Appendix

Data elements retrieved from Dataline query results

Category	Element	Variables / description
Patient characteristics		
	sex	male; female
	date of birth	
	age at start of second therapy	in years
	cancer type	
	survival status	alive; dead
	date of death / last follow up	
	survival time	in months, start of second treatment to date of death/last follow-up
Therapy		
	therapy timing	concurrent; closely timed (≤ 4 weeks); sequential (> 4 weeks interval)
	time interval	in months, (concurrent: start date second treatment - start date first treatment; sequential: start date second treatment - end date first treatment)
	first treatment	RT; immunotherapy
Immunotherapy		
	immunotherapy category	PD-1; PD-L1; CTLA-4; combination (both PD-1/PD-L1, CTLA-4)
	received drug on protocol	yes; no
	drug name for first (and second, if applicable) drug	
	no. of doses	
	total dose	in mg
	treatment start date	
	treatment end date	
Radiation therapy		
	RT technique	palliative; SBRT; other
	planned fractions	
	delivered fractions	
	planned dose	in cGy
	delivered dose	in cGy
	irradiated lesion	
	RT laterality	left; right; mediastinal
	RT site	mediastinum; hilum; upper lobe; lower lobe
	treatment start date	
	treatment end date	

Category	Element	Variables / description
Further therapy other that relevant course		
	other immunotherapy	yes; no
	timing other immunotherapy	before; after relevant course
	other RT	yes; no
	timing other RT	before; after relevant course
Adverse events (occurring after start of second treatment)		
	pneumonitis	yes; no
	CTCAE grade pneumonitis	
	attribution pneumonitis	to RT or immunotherapy: definitely; probably; possibly; unlikely; unrelated; unknown
	esophagitis	yes; no
	CTCAE grade esophagitis	
	attribution esophagitis	to RT or immunotherapy: definitely; probably; possibly; unlikely; unrelated; unknown
	dermatitis / rash / pruritis	yes; no
	CTCAE grade dermatitis / rash / pruritus	
	attribution dermatitis / rash / pruritus	to RT or immunotherapy: definitely; probably; possibly; unlikely; unrelated; unknown
	fatigue	yes; no
	CTCAE grade fatigue	
	other pulmonary AEs	yes; no
	CTCAE grade other pulmonary AEs	
	other gastrointestinal AEs	yes; no
	CTCAE grade other gastrointestinal AEs	
	other infections / sepsis	yes; no
	CTCAE grade other infections / sepsis	
	Other toxicity	renal/urinary dysfunction; thyroid dysfunction; other