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Treatment response biomarkers: working towards personalised radiotherapy for lung cancer

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Abstract

Due to major advances in the field of radiation oncology, patients with lung cancer (LC) can now receive technically individualised radiotherapy treatments. However, in the era of precision oncology, radiotherapy-based treatment selection needs to be improved as many patients do not benefit or are not offered optimum therapies. Cost-effective robust biomarkers can address this knowledge gap and lead to individuals being offered more bespoke treatments leading to improved outcome. This narrative review discusses some of the current achievements and challenges in the realisation of personalised radiotherapy delivery in patients with LC.

Introduction

LC remains the leading cause of cancer-related mortality with approximately 1 in 5 patients surviving to 5 years (1). Over half of all patients with LC receive radiotherapy and this treatment modality has an important therapeutic role in both curative- and palliative-intent settings (2). Response to radiotherapy is governed by complex interactions between hypoxia, DNA, genes, proteins, the immune system and cell repair and death pathways. Understanding how these interactions independently influence outcome is integral before identifying any clinical applications (3).

In the early-stage LC setting, complex multi-modality treatments are increasingly offered to patients who are not candidates for surgery. This includes different types of radiotherapy (including dose and fractionation) and whether systemic anti-cancer therapy (SACT) is offered. Multi-modality

treatments improve outcomes but are generally associated with higher rates of toxicity. Decision-making regarding the use of these treatments is based upon a limited number of clinical features that are associated with clinical prognosis and include performance status, comorbidities, stage, volume and location of disease.

Clinical decision making in the real-world setting is particularly challenging as groups of patients (such as the elderly, frail and those with comorbidity) are typically excluded or underrepresented in practice defining clinical trials. The availability of prognostic and predictive biomarkers would assist decision-making and therefore be extremely valuable to both patients and physicians. Prognostic biomarkers offer insights into patients' expected outcomes irrespective of the treatment they receive, while predictive biomarkers indicate the potential impact of a specific therapeutic intervention.

In contrast in the advanced LC disease setting, a number of drug treatments are selected based on tumour genetic information (known as genomic biomarkers, such as EGFR status) and biomarkers reflecting the tumor immune microenvironment (such as tumor PD-L1 status). This has led to more personalised treatments and improved outcomes.

Biomarker type	Potential application in lung cancer radiotherapy
Diagnostic	• To accurately predict key pathological information and reduce the reliance on solid organ biopsy. • To differentiate between tumours that have radiosensitive and radioresistant phenotypes.
Management	• To select optimal radiotherapy regimen, including type of radiation, dose and fractionation. • Improve radiotherapy target volumes by either improved tumour delineation or by identifying areas of local occult disease, e.g. mediastinal lymph nodes. • To support cytotoxic enhancement decisions about concurrent systemic therapies to enhance radiotherapy effect locally. • To support spatial cooperation decisions around concurrent systemic therapies to treat micro-metastatic disease or to induce abscopal effect. • To identify which patients will benefit from consolidation immunotherapy. • To predict prognosis to support discussions around cure and futility of treatment. • To predict local and distant tumour control. • To predict risk of acute and late toxicity. • To build decision support tools that generate personalised treatment plans and describe outcomes.

Follow up	• To reduce reliance on solid organ biopsy during disease monitoring. • To identify those patients with minimal residual disease earlier with the aim of offering treatments that will alter disease trajectory. • To differentiate between evolving radiotherapy-related fibrosis and local treatment failure. • To identify tumour control earlier to facilitate earlier discharge and identify patients who require more intense follow up.

Table 1: Potential applications for biomarkers to support patients with lung cancer undergoing radiotherapy.

There are currently no widely accepted tumor-, blood-, or imaging-based biomarkers that are used in the decision to offer radiotherapy (with or without SACT). The only exception is the European Medicines Agency's (EMA) decision to license consolidation durvalumab after concurrent chemo-radiotherapy in Europe for patients with PD-L1≥1% non-small cell lung cancer (NSCLC) (4).

The potential application of biomarkers to support LC radiotherapy is wide ranging and include supporting decisions around diagnosis, management and follow up (see table 1 for a summary). These biomarkers arise from a range of distinct scientific disciplines and technologies. A summary table is included for reference in the appendix (see table A.1) and some of the key advantages and disadvantages are summarised in figure 1.

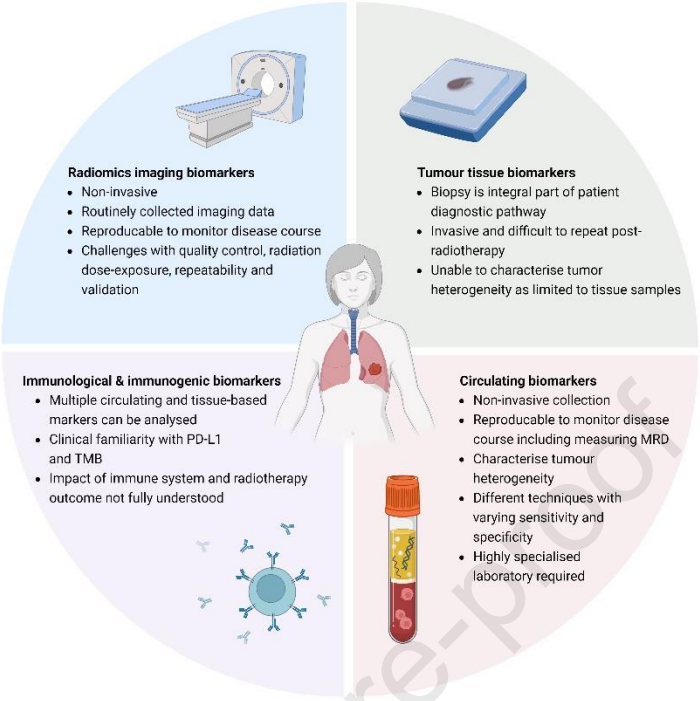

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Figure 1: Key advantages and disadvantages of biomarker technologies

In this narrative review, we will discuss current LC radiotherapy biomarker research, focusing on tumour-based, immunological, circulating and imaging-based biomarkers and their role in the evaluation of treatment response. Key studies, recent publications, trials in progress and future directions will be considered and are summarised in appendix table A.2.

Biomarkers used to predict toxicity and tumor hypoxia are beyond the scope of this review and are discussed in other review articles (5).

Genomic biomarkers

Several gene panels, called radiosensitivity indices (RSIs), can be used to predict tumour response to radiotherapy. The most validated RSI was developed using in-vitro cancer cell lines using a 10 gene panel to predict response to different radiotherapy regimens (6). Genes include recognised oncogenes and tumor suppressor genes involved in regulating cell proliferation, such as ABL1, PKC, RELA, CDK1 and IRF1 (7). They also include those with anti-apoptotic effects, such as JUN and HDAC1 and those involved in cancer inflammation and immune response, such as

RELA and STAT1 (7). It seems logical that mutations in these genes could result in cells that are more resistant to radiotherapy. CDK1 in particular is a gene implicated in anti-cancer treatment resistance (8).

It is suggested that RSIs could be used to identify a genomically adjusted radiation dose that is biological effective for individual tumors. This could help identify those patients with radioresistant tumors who would benefit from the addition of a radio-sensitising agents or those who might be better managed with surgery. There is also some evidence to suggest that patients with radioresistant tumours might be more sensitive to immunotherapy and this should be explored in prospective studies (9). Despite potential clinical applications, there is currently no prospective in-vivo validation of these indices to support their use in an interventional study.

Other research has focused more specifically on single gene mutations, such as DNA repair protein genes, e.g. ERCC1/2, or driver mutations. ‘ERCC1/2 are key proteins involved in the nucleotide excision repair pathway (7). ERCC1/2 single-nucleotide polymorphisms are associated with improved DNA damage repair of tumor cells and therefore are associated with radio-resistance and also resistance to platinum-based chemotherapy (10).’ A previously developed ERCC1/2 single-nucleotide polymorphism radiosensitivity signature used to predict response to radiotherapy has been validated in a cohort of RTOG0617 trial patients (10). Patients identified as having radiosensitive tumors experienced longer overall survival (OS) than those patients with the radioresistant phenotype.

Driver mutations, including EGFR, KRAS and ALK rearrangements, are associated with increased radiosensitivity, although the mechanisms are not fully understood (11,12). EGFR mutations appear to be associated with defective non-homologous repair pathways which prevents repair of radiotherapy-induced double-stranded DNA breaks (7). A systematic review describing studies where patients were treated with a combination of radiotherapy and tyrosine-kinase inhibitors (TKIs) describes increased toxicity with no clinical benefit (13). However, the studies summarised in the review did not use driver-mutation in their eligibility criteria likely obscuring any benefit.

EGFR mutations seem to be indicative of insensitivity to immunotherapy. There is also evidence of increased pneumonitis risk when TKIs are given following immunotherapy (14). As a result the European Society for Medical Oncology have recommended against the use of consolidation durvalumab in patients with EGFR mutant PD-L1 positive NSCLC (15). Radiotherapy-

TKI combination studies in patients with driver-mutation are ongoing with the aim of assessing safety and efficacy in both radical and palliative settings (16,17).

Proteomic and Metabolomic biomarkers

Protein and enzyme function affects response to radiotherapy. The inter-cellular enzyme Indoleamine-2,3-dioxygenase (IDO) converts Tryptophan (Trp) into Kynurenine (Kyn) (18). IDO mRNA is overexpressed in LC and increased activity is associated with inferior survival following radiotherapy, immunosuppression and immunotherapy resistance (18,19). Radiotherapy treatment resistance appear to be related to the reduction in reactive oxygen species and inhibition of CD8+T cells. Activity can be indirectly monitored using serum Kyn:Trp ratios. IDO inhibitors are being investigated in early-phase non-radiotherapy studies and could improve radiotherapy outcomes in patients whose tumors exhibit radiotherapy resistant through increased IDO activity (20).

Poly-ADP-ribose polymerase (PARP) is a DNA base-excision repair enzyme. PARP inhibitors have a role in the management of ovarian cancer, hormone-resistant prostate cancer and pancreatic adenocarcinoma. In these cancers, BRCA mutation is used as a marker of PARP inhibition sensitivity. BRCA mutations are associated with a deficiency in the repair of DNA double-strand breaks or homologous recombination, although other genes are implicated (21). BRCA mutations are rare in LC and are most commonly found in adenocarcinoma with an incidence of approximately 1% (22).

PARP inhibitors act synergistically with radiotherapy by increasing the risk of replication fork collapse resulting in double-stranded DNA breaks. Early-phase NSCLC studies suggest radiotherapy-PARP inhibitor combinations are tolerable with manageable toxicity and dose-escalation studies are ongoing, including the early phase CONCORDE platform study (23).

The optimum biomarker for PARP inhibition remains undetermined, but potential candidates include proteomic markers such as tumor PARP levels or genomic markers such as the identification of specific gene mutations. As BRCA mutations are rare in LC, a genomic composite marker could be more specific. Homologous recombination deficiency (HRD) scores have been developed and are an indirect measure of the cumulative amount of abnormal repair in response to previous double-stranded DNA breaks (24). A HRD score could also be a useful marker of radiosensitivity given the

direct relationship between double stranded DNA breaks and radiotherapy cell kill (25).

In SCLC, the recently identified POU2F3 non-neuroendocrine subtype may confer PARP inhibitor sensitivity and so may have a role as a treatment response biomarker (26). PARP inhibitor-radiotherapy combination studies with RT are underway (27).

Immunological and Immunogenomic biomarkers

Radiotherapy induced cell death is highly immunogenic and potentiates the anti-tumor immune response through several complex mechanisms. However the tumor micro-environment is typically immunosuppressive and dampens down the immune response, for example through the interaction between PD-1 and tumor PD-L1 receptors. Increased levels of PD-L1 are associated with worse survival following radiotherapy (28). In contrast, higher levels of circulating immune cells, such as CD8+Tcells, and tumour mutation burden (TMB) are associated with better outcomes following radiotherapy (29,30).

There is some evidence that tumor PD-L1 upregulation can occur during radiotherapy (28). Repeat biopsies to characterise these dynamics are not practical and can cause morbidity to patients. Therefore non-invasive biomarkers are required and these might include serum PD-L1 levels, circulating tumor cell (CTC) PD-L1 and circulating tumor DNA (ctDNA) TMB (28,31,32). In the palliative setting, PD-L1, CD8+Tcells and TMB independently predict immunotherapy response with composite measures appearing most sensitive (33). Currently there is no published data investigating these biomarkers in response to radiotherapy-immunotherapy combinations, but by extrapolating existing research, an immune-based composite biomarker might predict tumour control and immunotherapy benefit in patients undergoing combination treatment.

The post-hoc sub-group analysis of the PACIFIC trial data has led to the approval of consolidation durvalumab following concurrent chemoradiotherapy only in patients with PDL1>1% (34). This decision from the EMA remains contested as the analysis was unplanned and unpowered (35). It also does not align with the decision from other pharmaceutical evaluation agencies, such as the American Food and Drug Administration (36). Furthermore, over a third of the patients included in the study did not have PD-L1 analysis performed and the test was done on biopsies performed prior to chemo-radiotherapy. Outwith these logistical issues, PD-

L1 although commonly used, is an imperfect biomarker. Studies show that over half of all patients exhibiting PD-L1 expression greater than 1%, who subsequently receive durvalumab treatment, will eventually relapse. It also does not identify the third of patients who are cured by chemoradiotherapy alone and receive durvalumab unnecessarily.

In the advanced LC setting, the immune-driven abscopal effect is a phenomenon where treatment response is seen in non-irradiated tissue (37). Although the mechanism is not fully understood it appears to be related to radiotherapy CD8+T cell activation and despite being uncommon, is most often seen in patients receiving radiotherapy-immunotherapy combinations (38). The biggest series examining the abscopal effect in LC radiotherapy-immunotherapy treatments pooled the results of two negative studies. The results did not demonstrate an association between tumour PD-L1 and treatment response and other immunological markers such as CD8+T cells or TMB were not assessed. (39). A more complete larger trial is required to confirm these results and to better understand and define the abscopal effect, to identify predictive biomarkers and whether this phenomenon is inducible.

In the SCLC setting, chemotherapy-immunotherapy combinations are offered to patients with advanced disease. The recently identified immunotherapy sensitive YAP1 subtype is a potential treatment response biomarker to be explored in future radiotherapy-immunotherapy studies (26).

Circulating biomarkers

Circulating biomarkers, so called liquid biopsies, measured in the blood or tissue fluid are less invasive than tissue biopsy. They are repeatable and provide an acceptable method for longitudinal monitoring of treatment response. Serial analysis potentially enables a dynamic description of tumour heterogeneity and clonal evolution during a disease course, and minimal residual disease (MRD) following curative treatments (40). To date research has predominantly focused on describing technical aspects and significance of detection of ctDNA and CTCs, though alternative tumour components such as RNA and vesicles are also detectable.

Circulating tumor DNA:

Whole genome, exome and targeted next generation sequencing methods provide highly sensitive methods to identify ctDNA in the blood of patients

with LC undergoing radiotherapy. Current research has been summarised in several comprehensive review articles (41,42). The detection of ctDNA prior and following completion of radiotherapy appears to be associated with inferior survival and that post-radiotherapy detectable ctDNA/MRD predicts subsequent relapse four months earlier than standard-of-care imaging (43). ctDNA detection could also identify which patients might benefit from consolidation immunotherapy (43). This could allow patients with a negative ctDNA post chemoradiotherapy to avoid the risk of toxicity and additional treatment costs of immunotherapy. Gene sequencing can also be performed to investigate resistance mechanisms that might develop in response to treatment and identify future therapeutic targets.

Other researchers have described ctDNA dynamics during a course of radiotherapy. Frequently a release of ctDNA is seen during the first 72 hours of treatment (44). By sequencing this ctDNA genetic information can be assessed from the treated tumour. This presents an opportunity to gain genetic information from tumours in the curative setting when invasive biopsies are not pursued. In the metastatic setting ctDNA analysis could be used to characterise tumour heterogeneity and resistance mechanisms of progressive lesions after radiotherapy. Following radiotherapy, the release of ctDNA into the blood is presumably caused by tumor necrosis. It has been hypothesised that ctDNA detection could be used as a measure of underlying radiosensitivity with higher titres reflecting a more sensitive tumour (42).

Newer techniques, such as methylated cell free DNA (cfDNA) profiling have been shown to be sensitive and are cheaper than other techniques. In a landmark study, tumour-specific methylation patterns were assessed from blood samples taken from SCLC patients (45). Concentration of tumor methylated cfDNA levels were associated with survival. Serial analysis of the methylation profiles provided an opportunity to identify more aggressive phenotypes, describe response to treatment and could be used to personalise treatments.

Prospective trials are now required to compare sensitivity and specificity of different ctDNA detection methods, to confirm and better understand ctDNA dynamics and their relevance to radiotherapy-based treatments and outcomes. Current interventional trials include the phase 2 SCION study, where ctDNA titres following stereotactic ablative body radiotherapy are being used to guide consolidation immunotherapy (46). The APPROACH study is also underway and is using ctDNA dynamics to guide adjuvant almonertinib TKI following curative-intent radiotherapy for stage III EGFRm+ NSCLC (47).

Circulating tumour cells:

CTC concentration correlates with LC stage and disease burden and both baseline and post radiotherapy concentrations are prognostic of clinical outcomes (48–51). They are identified at lower frequency than ctDNA and detection assays need to be highly sensitive to be clinically useful. As a result current detection methods are not a sensitive measure of MRD and are less able to describe tumour heterogeneity and clonal evolution, particularly in patients with low volume and non-metastatic disease. Similar to ctDNA, CTC counts increase during a course of radiotherapy and this could potentially be exploited to increase diagnostic information about an individual's cancer (52).

The utility of CTCs in clinical practice is currently unknown. Research is needed to address whether CTCs represent a subpopulation of aggressive tumour cells and is a source of tumour seeding. Research efforts must also concentrate on understanding the significance of post-radiotherapy detection as some patients with detectable CTCs exhibit favourable clinical outcomes. Thus CTCs cannot be deemed a reliable marker of MRD.

Prospective studies that compare ctDNA and CTC analysis techniques are also required to identify their unique strengths and limitations with a view of identifying future roles for both. This includes validating detection thresholds and comparing their ability to accurately identify MRD following curative treatments.

<u>Imaging biomarkers</u>

Working within health systems with limited resource, the opportunity to use measurable features found in non-invasive routine imaging as potential biomarkers is attractive. Patients attend for multiple scans throughout their disease course, with CT scans being most frequent. CT scans demonstrate morphological features whilst PET-CT and MRI scans also describe biological features.

Within scans there is informative data that goes unexploited. For example, tumour dimensions and SUVmax do not acknowledge complex features such as shape, texture and contrast distribution. Radiomics presents an opportunity to automatically extract numerous features from imaging and through modern data methodology assign statistical significance to outcome data. In response to a growing number of retrospective low quality studies, guidance such as the Radiomic Quality Score and the Image

Biomarker Standardisation Initiative have been published to both critically appraise and improve the quality of future studies (53,54).

Radiomic-based imaging biomarkers:

Radiomic models may enhance diagnostic pathways, reduce reliance on invasive biopsy by identifying pathological subtypes, driver mutations and other features such as Ki-67 and as a result reduce time to definitive management (55,56).

Imaging features identified from radiomic studies could also play a role in designing more personalised radiotherapy treatment plans. An existing predictive model built using surgical specimens and pre-operative PET-CTs to identify occult regional lymph node metastasis could be adapted and validated to guide elective nodal radiotherapy to at risk areas (57). Benefit from additional mediastinal radiotherapy would need close evaluation against additional toxicity risk, particularly cardiotoxicity.

Radiomic studies have primarily focused on pre-treatment imaging to identify prognostic features. Integrating clinical data and semantic features into radiomic models enhances performance. A model built using the pre-SABR scans of patients with stage I-II NSCLC demonstrated improved performance by combining radiomic and semantic features, such as vessel attachment and pleural retraction, with Eastern Cooperative Oncology Group performance status (58). Another study included patients with stage III NSCLC undergoing chemoradiotherapy to build an actuarial deep-learning architectural model to predict tumour control (and pneumonitis risk) (59). The model combined features extracted from PET-CTs, serum cytokines and microRNA. It outperformed traditional tumour control probability models and performed well following validation which included 327 patients from the RTOG0617 study. Other researchers have described similar models that integrate radiomic and microRNA features and demonstrated improved performance, although in this study validation was limited to an internal cohort from a different time period (60).

The Maastricht-based radiomic research group published several studies applying their delta-radiomic/longitudinal analysis to weekly cone-beam CTs acquired during radiotherapy to verify target position. Unfortunately in their largest series, they were unable to validate a survival model built using the scans of patients diagnosed with stage I-IV NSCLC receiving curative-intent radiotherapy (61). Other researchers have used multitask learning methods and analysed different imaging modalities to improve model performance. This included the imaging acquired during the FLARE-RT study that included baseline CT, PET-CT and perfusion SPECT scans

and mid-treatment PET-CT (62). This analysis included patients diagnosed with stage IIB-IIIB NSCLC receiving concurrent chemoradiotherapy. Features identified from PET-CT scans, outperformed those found on CT and perfusion SPECT and those models built using multitask learning methods performed better than those using conventional methods. Despite no validation dataset, the study highlights the value of using novel data methods and multimodality longitudinal scans to enhance performance of prognostic models.

Radiomics analysis has a role in selecting optimum treatment combinations. A model that predicts pathological complete response (pCR) was built using pre- and post-neoadjuvant chemoradiotherapy PET-CT scans (63). All patients were diagnosed with stage III NSCLC and went onto have surgical resection of their disease. pCR was predicted in 93.4% and outperformed conventional PET-CT measures and radiologist assessment. This model could be adapted for patients being considered for tri-modality treatments where accurately predicting pCR after chemoradiotherapy may allow some patients to avoid surgery.

Radiomic analysis is also being used to non-invasively describe the tumor micro-environment. A study analysing tumour and peri-tumour regions identified features in both that outperformed PD-L1 in predicting response to consolidation immunotherapy after chemoradiotherapy as well as features prognostic for survival (64). An ongoing study includes a prospective and retrospective cohort that are aiming to identify features predictive of consolidation immunotherapy response (65). In recognition of the multiple, unvalidated radiomic studies published, it will compare performance of several models. Other models have been built that identify immune-inflamed tumour micro-environments by predicting the degree of CD8+Tcell infiltration. In one example, stage IV NSCLC patients receiving SABR-immunotherapy combinations responded better to treatment if their tumours were inflamed at baseline (66).

Despite more than a decade of radiomics research, currently no published model appears robust enough to be integrated into a randomised study and high-quality, transparent, validated, prospective studies are required.

Interventional studies integrating imaging biomarkers:

The LARTIA trial is the only published interventional study identified that used CT-based imaging features to offer patients an adaptive radiotherapy approach (67). All patients were diagnosed with stage III NSCLC treated with concurrent chemoradiotherapy. Patients whose tumours shrank on weekly CT scans had their radiotherapy replanned to reduce treatment

volumes. Only a quarter of the 217 patients required a replan and there was no control arm. The researchers reported this approach reduces toxicity rates without compromising control rates. A more targeted approach, such as integrating a model that predicts changes in tumour volume at baseline (68), could improve patient selection for future adaptive radiotherapy studies.

Five published PET-CT-based interventional studies were identified. Two of the studies used baseline imaging features to offer dose escalation one using FDG PET and SUVmax (69), the other F-MISO thresholds to quantify hypoxia (70). The other studies offered dose boosts based on mid-radiotherapy SUVmax (71–73). These studies demonstrate that dose modification and adaptive radiotherapy are technically achievable. However only one study met its primary endpoint of overall survival (71). Furthermore there are concerns that dose escalation is associated with increased toxicity. The F-MISO study results supported that hypoxic tumours are radioresistant and associated with poor radiotherapy outcomes.

There are ongoing interventional studies integrating imaging biomarkers, including the SPRINT study that offers patients diagnosed with stage II-III NSCLC ≥50% PD-L1 induction pembrolizumab and 'dose-painted' radiotherapy (74). Radiotherapy dose offered is dependent on the metabolic tumor volume seen on post-immunotherapy PET-CT with smaller lesions receiving lower doses.

Conclusions and Future perspectives:

Radiotherapy is an important treatment modality offered to numerous patients diagnosed with LC. This review has summarized research elucidating potential prognostic and predictive biomarkers, poised to aid in informed decision-making for patients. The goal is to empower clinicians and patients to engage in realistic discussions regarding treatment expectations, associated risks, and potential treatment. Ultimately, the objective is to equip patients and clinicians with the necessary tools to make personalized and well-informed decisions around their care.

Non-invasive biomarkers, such as those from blood tests or imaging, are particularly attractive as they could reduce the reliance on invasive biopsies. This could benefit select patient groups by reducing the time from presentation to starting treatment. They could also be used to describe tumor heterogeneity, clonal evolution and identify treatment resistance

mechanisms. This information is not only useful at diagnosis but could also be used to provide enhanced disease monitoring following treatment.

To address the limitations in the current published research on biomarkers in the field of LC radiotherapy, well designed large prospective studies are required. These studies should integrate multiple health technologies in order to better describe the significance, strengths and limitations of novel biomarkers. Moreover, it is crucial to incorporate traditional prognostic and predictive features such as tumor volume and patient performance status into these analyses. This approach will ensure that any novel biomarker is evaluated in conjunction with established parameters, enabling assessment of its additive value in clinical decision-making. In addition, assessing biomarker validity using independent patient cohorts is a necessary step in demonstrating robustness of these analyses.

Researchers should utilise guidelines on the use of biomarkers when designing and carrying out research in order to improve the overall quality of their studies (75,76). They should also make their protocols and results publicly available to encourage research collaboration, transparent discussion of their findings and to demonstrate the quality assurance processes used.

To increase the chance of clinical impact, several important concepts should be considered in studies integrating biomarkers. Ensuring that eligibility criteria allow for the inclusion of patients who are representative of the general lung cancer population is crucial for the validity and applicability of clinical research findings. Considering cost and cost-effectiveness is also essential in the design and implementation of biomarkers within studies, given the cost of biomarker technologies.

Finally, there is an unmet need to integrate biomarker research with modern data science methodologies. Techniques, such as machine learning, can analyse a large amount of clinical and biomarker data from a range of disciplines. They can generate complex models predicting outcomes with enhanced accuracy and delivering realistic outputs. This, in turn, presents an opportunity to integrate biomarker-based models into decision support tools, with the goal of enhancing personalized decision-making (76). Well-designed, user-friendly decision support tools can present treatment choices, trade-offs as well as predict outcomes in visual displays. Proof of concept and acceptability already exists with the widely adopted Predict Breast Cancer tool (77). Predict Breast Cancer is used by clinicians to aid discussions around adjuvant therapies in patients with breast cancer. The model includes ER, HER2/ERRB2 and Ki-67 status,

which are known prognostic and predictive biomarkers for breast cancer treatment.

In summary, despite the wealth of published exploratory research and pressing clinical need, no biomarker has yet gained full acceptance and integration into clinical practice for LC radiotherapy. However, there is clear potential and ongoing research interest in the development of biomarkers that can significantly enhance decision-making and patient outcomes. Personalised treatments in lung cancer radiotherapy remains an aspiration, with the identification of robust biomarkers and predictive modeling representing the crucial first step.

Appendix A:

Biomarker 'omic':	Description:	Analysis methods:	Potential application in lung cancer radiotherapy:
Genomic biomarkers	Genetic analysis and sequencing of tumor or normal tissue material, usually from solid organ biopsy. Includes DNA, RNA (transcriptomics), SNP and epigenetic analysis. Single gene or multiple genes can be analysed. Hereditary and somatic mutations can be assessed.	Techniques include whole-genome, whole-exome and methylation-specific next generation sequencing methods. Targeted gene panels. SNP array.	**Tumor-based:** genetic-based radiosensitivity analysis that suggests a genomically-adjusted personalised radiotherapy dose. **Tissue-based:** assess an individual patient's risk of radiotoxicity. Predicts those who would benefit from a radioprotective agent.
Circulating biomarkers: Liquid biopsy	Non-invasive genomic analysis using tumor material isolated in blood or tissue fluid. Analysis can include circulating DNA, RNA and vesicles. Describes tumor heterogeneity and, through serial assessment, clonal evolution.	Techniques include whole-genome, whole-exome and methylation-specific next generation sequencing and more targeted gene panels.	**Tumor based:** replace invasive tumor biopsies by non-invasively identifying relevant tumour pathological characteristics. Post-radiotherapy ctDNA detection as a marker of minimal residual disease and used to prognosticate patients and to predict those who will benefit from adjuvant therapies.

Proteomic biomarkers	Protein and enzyme analysis of tumor or normal tissue material. Includes structure and function analysis and concentration titres.	Techniques include protein microarray, mass spectrometry and protein assay. .	**Tumor-based:** analysis of proteins associated with radiosensitivity, e.g. PARP. Identify those who benefit from medications that enhance radiotherapy effect. **Tissue-based:** analysis of proteins associated with radiotoxicity. Identify those who would benefit from the addition of a radioprotective agent.
Metabolomic biomarkers	Metabolism products or substrate analysis to indirectly measure protein function.	Metabolite and substrate assay.	**Tumor-based:** similar to proteomics but focused on cell metabolism. **Tissue-based:** similar to proteomics but focused on cell metabolism.
Immunological and immunogenomic biomarkers	Recognising the complex relationship between tumor and the immune system in both tumour development, Analysis of cytokines, immune cells and immune markers that reflect underlying immune system functioning. Includes immunogenomics which includes the analysis of mutant tumor-originating peptides and immune-related genes that are involved in immune response.	Techniques include cytokine assays, immune cell subtyping and immune cell protein assays.	**Tumor-based:** describing the dynamic relationship between radiotherapy effect, tumor and the immune system. Identify those who benefit from consolidation immunotherapy. **Patient-based:** radiotherapy associated lymphopenia associated with increased mortality.
Radiomic-based imaging biomarkers	Using data algorithms to extract complex spatial features from imaging, e.g CT, PET-CT and MRI, and to correlate with diagnostic, predictive and prognostic outcomes.	Multi-step process including image acquisition, image segmentation, feature extraction and qualification and analysis.	**Tumor-based:** replace invasive tumour biopsies by non-invasively identifying relevant tumor pathological characteristics. Identify imaging features that prognosticate or predict benefit from adjuvant therapies. **Tissue-based:** identify imaging features associated with risk of radiotoxicity, such as pneumonitis or cardiac toxicity.

Dosiomic biomarkers	Using data algorithms to extract complex spatial features from radiotherapy dose distribution and to correlate with predictive and prognostic outcomes.	Multi-step process like radiomics but using radiotherapy dose distribution instead of scans.	**Tumor-based:** identify dose distribution to the tumor volume that can predict response to radiotherapy. **Tissue-based:** identify dose distribution features associated with risk of radiotoxicity, such as pneumonitis or cardiac toxicity
Patient reported outcome measure biomarkers	Using serial assessment of a patient's symptom burden, functioning and quality of life to look for changes in scoring over time that correlates with a chosen outcome	Using validated questionnaires, e.g. EORTC QLQ-C30 and lung cancer specific EORTC QLQ-LC13	**Tumor-based:** identifying trends in reported symptoms and quality of life to predict treatment response or tumor progression. **Patient-based:** identifying trends in reported symptoms and quality of life to predict toxicity early.
Multiomic/Panomic biomarkers	Using data algorithms to combine 'big data' from a range of biomarker disciplines to discover complex associations and develop predictive models.	Using modern data algorithms such as machine learning.	As per previous potential applications.

Table A.1: Description of different types of biomarker

Study	Arms	Patients	Median follow up	Primary end point	Primary results	Biomarker	Comments
Genomic biomarker studies:							
RTOG0617 cohort analysis (10) **Retrospective analysis of patients from a phase III study**	Using a cohort that received 60Gy/30# from the dose escalation RTOG06117 study to validate a previously developed ERCC1/2 DNA repair gene SNP signature as a radiosensitivity biomarker.	Retrospective analysis of RTOG0617 dose escalation trial. N=275 analysed NSCLC Stage III	28.7 months	To externally validate the ERCC1/2 radiosensitivity biomarker	Radioresistant cohort associated with worse OS. OS: HR=1.4; 95%CI, 0.96-2.01, p=0.076	ERCC1/2 gene signature.	Only published in abstract form. No prospective validation available. Model can also be used for normal tissue radiosensitivity.
Genomic biomarker studies in progress:							
The STEREO study(16) **Phase II**	Single arm: Osimertinib + risk-adapted SABR to patients with synchronous oligo-metastatic EGFR mutant NSCLC	Aiming to recruit N=60 NSCLC Stage IV EGFRm+ (exon 19 deletion +/- exon 21 L858R)		Safety of combination treatment. If safety proven, efficacy tested as PFS.	Awaited	EGFR mutation as entry criteria.	No control arm so difficult to make efficacy comments. HALT study similar but using SABR to areas of oligo-progression in patients established on TKI.
NCT04636593 study (17) **Phase II**	Single arm: almonertinib + radical radiotherapy. If V20≥28% at planning patients offered 2 months induction almonertinib to downsize prior to RT to meet dose constraint.	Aiming to recruit N=43 NSCLC Stage III EGFRm+		Incidence of radiation pneumonitis grade≥3 within 6 months of radiotherapy	Awaited	EGFR mutation as entry criteria.	No control arm so difficult to make efficacy comments. The AENEAS trial has demonstrated improved PFS with almonertinib vs gefitinib in the stage IV setting (HR, 0.46; 95%CI, 0.36-0.60; p<0.001) with a similar toxicity profile.
Proteomic and Metabolomic biomarker studies:							
Zhu et al (18) **Prospective exploratory study**	Non-intervention study: Measuring IDO activity pre-RT and one-week post-RT through	N=104 NSCLC Stage I-II: 42 Stage III-IV: 62	20.8 months	To describe the association between IDO activity and clinical outcomes	On multivariate analysis:	Kyn:Trp ratios.	Higher BED (≥70Gy) associated with activation of the immune system.

	measuring serum Kyn and Trp levels.	Chemotherapy given: 53			Raised pre-RT Kyn:Trp ratios associated with shorter PFS: HR=1.74; 95%CI, 1.00-3.03, p=0.049. Raised post/pre-RT Kyn:Trp ratios associated with improved OS HR=0.48; 95%CI, 0.24-0.99, p=0.045.		Results suggest that IDO activity could be used as a marker to adjust RT dose or used to predict benefit from consolidation immunotherapy.
The CONCORDE trial (23) **Phase Ib**	Five arms: 3:1 randomisation between radical RT+/- DDRi 2 arms open to recruitment: Olaparib AZD1390 (an ATM inhibitor) Future arms to include consolidation IO.	Aiming to recruit N=200 (40 in each arm) NSCLC Stage IIB-IIIC. Patients allowed sequential CRT approach	2 years	To determine the recommended phase II dose and safety profiles of different DDRis	Awaited	Planned sub-analysis may identify potential biomarker signals.	Use of the innovative TiTE CRM design to identify the RP2D for each DDRi. Control arm present which allows for safety assessment as well as some efficacy. The 40 patients receiving radiotherapy alone provides opportunity to describe toxicity profile of patients.
NCT03532880 study (27) **Phase I**	Single arm: Low dose thoracic radiotherapy (30Gy/10#) + Olaparib.	Aiming to recruit N=26 Extensive stage SCLC following completion of 4-6 cycles of platinum-etoposide.	1 year	Maximum tolerated dose and safety of olaparib in combination with low dose thoracic radiotherapy	Awaited	No specific biomarker.	If olaparib is shown to be safe, POU2F3 subtype could be used as inclusion criteria for subsequent randomised studies.
Immunological biomarker studies:							
The PACIFIC trial (34) **Phase III**	Two arms: CCRT +/- consolidation durvalumab	N=713 NSCLC Stage III.	34.2 months	Clinical outcomes: OS and PFS	Improved OS and PFS seen in those patients receiving consolidation durvalumab.	Tumor PD-L1≤1%.	No benefit to OS seen in those with PD-L1≤1% or with EGFR mutation or ALK rearrangement.

					OS: HR=0.72; 95%CI, 0.59-0.89, no p value PFS: HR=0.55; 95%CI, 0.45-0.68, no p value		Only recent practice changing study with improvement in overall survival in stage III NSCLC.
Theelen et al (39) **Pooled analysis of PEMBRO-RT Phase II and MDACC Phase I/II trials.**	Both studies had 2 similar arms: Pooled arm A: pembrolizumab alone Pooled arm B: pembrolizumab + SABR (24Gy/3# or 50Gy/4#) or RT (45Gy/15#)	N=148 NSCLC Stage IV With at least one unirradiated lesion to monitor for abscopal (out-of-field) response.	33 months	PEMBRO-RT: Improvement in overall response rate at 12 weeks. MDACC: Best abscopal (out-of-field) lesion response rate (ARR)	Improvement in ARR (and PFS and OS) seen in those who received radiotherapy. ARR: OR-2.96, 95%CI 1.42-6.20, p=0.0039.	No association between outcome and tumor PD-L1.	Tumor PD-L1 did not influence outcome. Pooled analysis needs validating in larger study with other immune biomarkers, such as TMB and CD8+T cells.
Circulating biomarkers: ctDNA							
Chaudhuri et al (40) **Retrospective exploratory study**	Exploratory retrospective analysis of longitudinal blood tests taken before and after lung cancer RT.	N=40 NSCLC:37 SCLC: 3 Stage I-II: 14 Stage III: 26	Not described.	Analysing association of ctDNA MRD with freedom from progression.	94% of those who progressed, ctDNA was detected on first postRT blood sample	ctDNA.	Progression identified a median of 5.2 months earlier than on imaging. 53% of patients were found to have druggable mutations from ctDNA analysis.
Moding et al (43) **Retrospective exploratory study**	Exploratory retrospective analysis of longitudinal blood tests taken before and after lung cancer CCRT +/- consolidation immunotherapy	N=65 NSCLC Consolidation immunotherapy: 28	Not described	Patients with post CCRT ctDNA MRD whose ctDNA concentrations reduced during consolidation immunotherapy would do better than those with ctDNA MRD who did not receive consolidation immunotherapy	Patients with ctDNA MRD who received immunotherapy than those who did not and those with reducing ctDNA concentrations appeared to do better compared to those with increasing concentrations.	ctDNA.	Patients with negative ctDNA post-RT had good clinical outcomes – although one such patient died of immunotherapy pneumonitis.
ctDNA dynamics (44) **Prospective exploratory study**	Non-intervention study: Assessing ctDNA before during and after RT	N=11 NSCLC: 9 No tissue: 2 Stage I-II: 7	Not described	Not described	91% of patients showed temporary increase of ctDNA	ctDNA.	Using mouse models demonstrated that by targeting implanted tumours with radiotherapy increased

		Stage III: 4			within 72 hours after initiation of RT.		ctDNA concentrations and that through sequencing this DNA were able to describe genetic detail about the tumour. Suggests ctDNA analysis may benefit those patients where biopsy not possible.
Small Cell ctDNA (45) **Retrospective exploratory study**	Non-intervention study: cfDNA-methylation profiling using bloods at baseline and7 patients had post-treatment samples taken.	N=78 SCLC Limited stage: 29 Extensive stage: 49	Not described	Exploratory	Tumor methylation patterns were detectable even in patients with a low turmor burden and correlated with stage and OS. Patterns identified SCLC subtypes.	cfDNA tumour-specific methylation.	Suggests cfDNA methylation profiling could be used to detect, monitor and subtype SCLC.
Circulating biomarkers: ctDNA studies in progress:							
SCION study (46) **Phase II**	Single arm: Patients offered SABR + C4 durvalumab. ctDNA then assessed: • Negative: no further treatment Positive: Randomised to no further treatment vs C8 durvalumab	N=94 NSCLC Stage I-II		Relapse rate at 18 months	Awaited	ctDNA.	Comparing primary outcome with historic controls, although does have groups within the study to do comparisons. True biomarker driven study where treatment influenced by detection of ctDNA
APPROACH study (47) **Phase II**	Four arms (arms A+B surgical). All patients receive almonertinib 8 weeks induction therapy. Patients randomised between arm C+D after radical radiotherapy: Arm C: receive almonertinib for 2 years.	N=156 NSCLC Stage III		Objective response rate	Awaited	ctDNA.	True biomarker driven study where treatment influenced by detection of ctDNA

	Arm D: receive almonertinib if ctDNA positive, can restart if becomes positive after a period of being negative.						
Circulating biomarkers: CTC							
Chinniah et al (49) **Prospective exploratory study**	Serial CTC analysis before and after CCRT.	N=48 NSCLC Stage II: 6 Stage III: 42	10.9 months	To assess CTCs as a biomarker	Those with detectable CTC post-RT went onto relapse (median lead time=6.2 months range (0.1-12.0 months)).	CTC.	25% of relapses had no detectable CTC, suggesting that current detection sensitivity is not robust enough for clinical use.
Fernandez-Gutierrz et al (50) **Sub-analysis of phase III**	Baseline CTC analysis before CCRT.	N=79 SCLC Limited stage	Not described	OS	CTC concentration associated with survival with ≥15 CTC the most significant threshold. OS 6.0 months vs 30.8 months (p<0.001)	CTC.	Only published in abstract form. Patients from a single centre.
Deng et al (51) **Prospective exploratory study**	Serial CTC analysis before and after PCI. All patients had CCRT.	N=20 SCLC Limited stage: 11 Extensive stage: 9	39.2 months	PFS and OS.	After PCI, patients with ≥4 CTC did significantly worse than those with <4. PFS 28.1 months vs not reached, (p=0.001). OS not reached vs not reached, (p=0.029)	CTC.	Those patients with a quicker decline in CTC post PCI experienced improved PFS and OS. 3/9 limited stage and 4/11 extensive stage relapsed within the follow up period which appears to be a low relapse rate.
Martin et al (52) **Prospective exploratory study**	Serial CTC analysis before and during radical or palliative RT.	N=27 NSCLC Stage I: 2 Stage II: 2 Stage III: 5 Stage IV: 17	No follow up beyond RT described.	To determine whether RT mobilises viable tumour cells into the circulation.	Increased concentration of CTCs detected in 7/9 palliative and 4/8 radical patients during RT.	CTC.	Concern that CTC mobilisation during RT could increase risk of tumour metastasising and could be used a biomarker for change

							in fractionation or systemic therapies.
Interventional imaging-based biomarker studies: CT based							
LARTIA trial (67) **Phase II**	Single arm: CCRT • Weekly replans in those patients with tumour shrinkage	N=217, only 50 required replans NSCLC Stage III.	22.8 months	To reduce acute and late G3+ pulmonary toxicity compared to historical cohort (RTOG9410).	Compared to historic controls reduced pulmonary toxicity. LARTIA – acute: 2%, late 4%. RTOG9410 – acute: 13%, late 17%. Observed as reduction in toxicity, no p value or CI attached.	Tumor shrinkage during radiotherapy.	Compared to historic controls reduced no impact on local failure rates. Only 23% suitable for replanning.
PET-based							
PET-Boost trial (69) **Phase II**	Two arms: Whole tumour group - 78Gy/24# to entire tumour. PET-sub-volume group - 84Gy/24# with dose escalation to high FDG uptake region within tumour. Lymph nodes treated 66Gy/24# in both arms	N=107 NSCLC Stage II = 13 Stage III = 94 CCRT= 77 Seq CRT = 10 Rad RT = 20	38 months	1-year freedom from local failure (FFLF)	Similar local control in both groups Whole tumour group: FFLF=97%; 95%CI, 91-100 PET-sub-volume group: FFLF=91%; 95%CI, 82-100	Tumor SUV.	Similar survival outcomes in both groups. No direct comparison to standard treatment but higher rates of local control compared to historical controls. Closed early due to slow accrual. G3+ acute and late toxicity rates high in both arms. 9 deaths possibly related to treatment.
RTEP-5 trial (70) **Phase II**	F-MISO PET-CT used to identify hypoxic tumours. Two F-MISO+ (hypoxic tumour) arms: CCRT Arm A - Mean dose 77.1Gy with boost to hypoxic.	N=52 NSCLC Stage Ib=2, II=3, III=48, IV=1. Hypoxic tumour=34 Non-hypoxic=20	14 months	Tumour response at 3 months	Hypoxic tumours do not benefit from dose escalation. F-MISO+: Arm A: 50%, 95%CI, 31-69%	Tumor F-MISO+.	Hypoxic tumours are associated with worse clinicals outcome (OS at 1y). Dose escalation to tumour subregions appeared safe.

	Arm B - Mean dose 66Gy F-MISO- (non-hypoxic tumour) arm: 66Gy				Arm B: 50% 95%CI, 24-76% F-MISO- arm: 70%, 95%CI, 48-85		
CRTOG1601 trial (71) **Phase III**	Two arms: Arm A – replan based on PET-CT at 18-20# with dose escalation ≥66Gy/30# (2.2-3.2Gy/10#). Arm B – 60Gy/30#	N=226 NSCLC Stage III CCRT+ consolidation chemotherapy	Not documented	Overall survival	Dose escalation associated with improved OS. Arm A: 44.6 months. Arm B: 28 months (p=0.001)	Tumor SUV.	Only published in abstract form currently. Dose escalation associated with improved PFS but not ORR. No difference in toxicity.
NRG-RTOG 1106/ACRIN 6697 (R1106) trial (72) **Phase IIR**	Two arms: Arm A – replan based on PET-CT at 40Gy with dose escalation up to 80.4Gy/30# (2.2-3.8Gy/9#). Arm B – 60Gy/30# with weekly carbo/paclitaxel.	N=127 NSCLC Stage III CCRT	3.6 years	Local-regional progression freedom at 2 years	No benefit to local-regional progression. Arm A: 54.6%, 95%CI, 39.9-67.0 Arm B: 59.5%, 95%CI, 37.9-75.7	Tumor SUV.	Only published in abstract form currently. No benefit to PFS or OS from dose escalation. G3+ oesophagitis higher in dose escalation arm (17.4% vs 5.0%). No evidence of increased cardiac/pulmonary toxicity.
Kong et al (73) **Phase II**	Single arm: Replan based on PET-CT after 40-50Gy with dose escalation up to total dose of 86Gy/30#. Median dose 83Gy (63-86Gy),	N=42 NSCLC Stage II=4 Stage III=38	47 months	2 year loco-regional tumour control (LRTC)	Compared to historical local unpublished controls, the researchers demonstrated improved local tumour control. Study LRTC: 62% Historic control LRTC: 34% 2%	Tumor SUV.	No true control arm. Real-world populations included: poor PS, weight loss and poor lung function included and the majority of patients (98%) received dose escalation. This study led to RTOG1106 trial – see below.
Prospective imaging-based biomarker studies in progress: **CT based**							

SPRINT study (74) **Phase II**	Two arms: Arm A: patients with tumour PD-L1≥50% offered C3 induction pembrolizumab followed by dose painted RT with lesions with metabolic tumor volume (MTV)>20cc receiving 55Gy/20#, smaller lesions 48Gy/20#. Followed by C12 pembrolizumab. Arm B: standard CCRT and adj therapy.	N=25 in dose painted arm. N=38 in control arm. NSCLC Stage II-III		Progression free survival	Uses PFS to investigate whether dose-painted RT+immunotherapy is safe and effective for patients whose tumour is PD-L1≥50%.	Tumor MTV	

Table A.2: Summary of studies integrating biomarker analysis.

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Thank you for your consideration.

Yours sincerely,

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