

# Blood-based biomarkers for precision medicine in lung cancer: precision radiation therapy

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**Abstract:** Both tumors and patients are complex and models that determine survival and toxicity of radiotherapy or any other treatment ideally must take into account this variability as well as its dynamic state. The genetic features of the tumor and the host, and increasingly also the epi-genetic and proteomic characteristics, are being unraveled. Multiple techniques, including histological examination, blood sampling, measurement of circulating tumor cells (CTCs), and functional and molecular imaging, can be used for this purpose. However, the effects of radiation on the tumor and on organs at risk (OARs) are also influenced by the applied dose and volume of irradiated tissues. Combining all these biological, clinical, imaging, and dosimetric parameters in a validated prognostic or predictive model poses a major challenge. Here we aimed to provide an objective review of the potential of blood markers to guide high precision radiation therapy. A combined biological-mathematical approach opens new doors beyond prognostication of patients, as it allows truly precise oncological treatment. Indeed, the core for individualized and precision medicine is not only selection of patients, but even more the optimization of the therapeutic window on an individual basis. A holistic model will allow for determination of an individual dose-response relationship for each organ at risk for each tumor in each individual patient for the complete oncological treatment package. This includes, but is not limited to, radiotherapy alone. Individualized dose-response curves will allow for consideration of different doses of radiation and combinations with other drugs to plan for both optimal toxicity and complete response. Insights into the interactions between a multitude of parameters will lead to the discovery of new pathways and networks that will fuel new biological research on target discovery.

**Keywords:** Lung cancer; biomarkers; precision medicine; radiotherapy

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## Background

Precision medicine aims to take into account individual variability in genes, environment, and lifestyle for each person in order to achieve the most accurate treatment and prevention. This is in contrast to the traditional “one-size-fits-all” or “standard” approach, in which treatments are given based on the overall response or tolerance in the whole patient population, with little consideration for individual differences. Typically, only a proportion of patients will be cured by the standard therapy (1,2). The concept of “precision medicine” may not be completely new. As an example, in radiotherapy for lung cancer, the anatomy of the patient and its relation with the tumor are delineated on an individual basis and the prescribed dose to the target volumes are often limited by constraints of the organs at risk (OARs) (3). However, it is clear that these physical parameters are only the beginning of the move to a more holistic view of a patient and disease.

It is compelling that biomarkers in the blood can reflect the status of a tumor and of the OARs to allow precision radiation dose prescription and adaptation during therapy. However, these blood biomarkers should not be viewed alone, but in the context of the anatomical deposition of the radiation dose in the tissue. Indeed, the extent of radiation damage is not only determined by the radiosensitivity of an OAR, but also by dose-volume parameters and the regional characteristics of the OAR (4). Both deterministic and stochastic factors play a role in the outcome of radiotherapy. Moreover, irradiation of functionally irrelevant parts of the lung without the capacity to function, such as emphysematous bullae, will not have an effect on toxicity, even in individuals who may be sensitive to radiation injury (5,6).

High doses of radiotherapy to the lung and heart have been reported to negatively correlate with survival (7,8). Although severe organ toxicities are life-threatening and there is a high possibility of unreported or unidentified grade 5 toxicities, most cases of death in non-small cell lung cancer (NSCLC) patients treated with radiotherapy may not be caused by direct organ toxicity, because the number of reported grade 5 toxicities is much less than the number of deaths without evidence of disease progression. Recently, accumulating evidence has supported the idea that the immune system may play an important role in tumor control and survival (9-12). The immune system is also susceptible to radiation damage, which may impact tumor control and survival. Therefore, survival may also serve as an endpoint of normal tissue toxicities of the

immune system that has not been clearly defined previously. Considering that a tumor originates from normal tissue, it may share some similar radiosensitivity traits with the normal tissue. Under such circumstances, biomarkers of the radiosensitivity of normal tissue could also partially reflect the radiosensitivity of the tumor. Using a predictive model that incorporates dosimetry to both tumor and normal tissue, radiosensitivity biomarkers can be applied to guide precision radiotherapy. Such a model can also be used to better identify radiosensitivity biomarkers, because otherwise, the biomarkers would be buried under the noise of dosimetric variations.

Therefore, an integral approach that combines biomarker detection, dosimetry, normal tissue preservation, and tumor control can be employed to identify and utilize biomarkers for precision radiotherapy. In this review, we will discuss blood-based biomarkers with an emphasis on the methodology for how these biomarkers can be identified and utilized in the integrated approach.

## Biomarkers for normal tissue complications

Radiation damage of the normal tissue is a major cause of radiation complications. Several studies support the hypothesis that normal tissue radiosensitivity is influenced by genetic features of the patient (13). Normal tissue radiation complications may also be influenced by immunological or inflammation responses to the injury (14,15). Therefore, in terms of functionality, both radiosensitivity and non-radiosensitivity biomarkers will be potentially associated with the normal tissue complication, while in terms of originality of the biomarkers, both genetic biomarkers [such as single nucleotide polymorphisms (SNPs)] and serum or plasma biomarkers (such as inflammation cytokines) will be potentially associated with radiation-induced lung toxicity (RILT), including radiation pneumonitis (RP) and pulmonary fibrosis. In addition, other factors, such as gender, body weight, life style habits, medication use, etc., also influence the risk and severity of side effects. In this section, we will discuss blood biomarkers for normal tissue complications in terms of: (I) genomic biomarkers; (II) proteomics biomarkers; (III) cytokines; and (IV) an integrated approach to identifying predictive markers.

### *Genomic biomarkers*

The majority of studies on genomic blood markers

investigated the association SNPs and radiation toxicity (13,16,17). SNPs associated with the side effects of radiation have been identified by high-throughput genotyping, through genome-wide association studies (GWAS), and by candidate gene studies (13). The candidate genes are usually those involved in DNA repair such as excision repair cross-complementing (ERCC), X-ray repair cross complementing (XRCC), and so on (18,19), in inflammation such as transforming growth factor beta 1 (TGF- $\beta$ 1) and tumor necrosis factor alpha (TNF $\alpha$ ), in oxidative stress, and in apoptotic pathways such as p53. Many studies have reported potential SNPs in these genes that are associated with radiation toxicities (20,21).

An SNP of the ataxia telangiectasia mutated (*ATM*) gene, a central mediator of the radioprotective machinery in response to radiation therapy that participates in cellular stress responses, control of cell cycle checkpoints, repair of double-strand breaks (DSBs), and initiation of apoptosis, has also been reported to be a risk factor for RP. Xiong *et al.* reported that *ATM* variants [e.g., rs189037 (-111 G) and rs228590T] independently and jointly have a substantial impact on the risk of severe RP in NSCLC patients treated with radiotherapy and also reported a consistent effect of the rs189037G/rs228590T/rs1801516G (G-T-G) haplotype (17).

SNPs of TGF- $\beta$ 1 or proteins in its pathway have been reported to have a significant influence on radiation toxicity. The *HSPB1* rs2868371 SNP was associated with grade 3 or greater RP in both training (P=0.031) and validation sets (P=0.025) (22). Interestingly, this SNP was also associated with the risk of grade 3 or greater radiation-induced esophagitis in both training (P=0.045) and validation cohorts (P=0.031) (23). The hazard ratio (HR) was approximately 0.25. In addition, the *TGF- $\beta$ 1* rs1800469 SNP was associated with a higher risk of radiation esophagitis in both training (P=0.045) and validation (0.023) cohorts with a HR of about 2.50 (23). Yuan *et al.* from the University of Michigan reported that genetic variation in the TGF $\beta$ 1 pathway genes may be associated with combined thoracic toxicity of lung, heart, and esophagus (16). TGF- $\beta$ 1-509 T allele carriers had significantly less severe radiation esophagitis (P=0.019) and a lower mean grade of radiation-induced thoracic toxicity (RITT) (P=0.009) than TGF- $\beta$ 1 CC patients. Patients with sensitive genotypes in this pathway had a significantly higher grade of toxicity in lung, esophagus, and heart than those without sensitive genotypes (P<0.01).

Yuan *et al.* discovered that patients with different genotypes of rs1982073:T869C in *TGF- $\beta$ 1* are at lower risk

of RP after radiation therapy for NSCLC (16) independent of dosimetric factors such as total lung volume receiving more than 20 Gy ( $V_{20}$ ) and mean lung dose (MLD). Patients with the CT or CC genotype in *TGF- $\beta$ 1* rs1982073:T869C had a significantly lower incidence of severe RP than those with the TT genotype, especially among patients who had received an MLD <20 Gy or a  $V_{20}$  <30%. Indeed, this SNP in *TGF- $\beta$ 1* could be used to separate patients into different risk groups even after correction for MLD. External validation, however, is not yet available for this finding.

Another SNP is the *TNF- $\alpha$*  0629:308 G > A AA genotype, which was shown to be associated with severe RP, although the number of patients in that study was small (24). Among four patients with the AA genotype, three (75%) experienced severe RP versus 21 of 92 patients (23%) with the AG/GG genotype. This difference could not be accounted for by differences in MLD.

DNA repair is important in radiation injury. Functional polymorphisms of the base-excision repair genes *XRCC1* and *APEX1* and genetic variants of the nonhomologous end-joining gene *LIG4* can predict the risk of RP, not only in Caucasians but also in Han Chinese patients (18,19,25). Furthermore, polymorphisms in vascular endothelial growth factor (VEGF) may modulate the risk of RP, and the CC genotype of *HSPB1* rs2868371 is associated with a higher risk of severe RP (22,26). Subsequent analyses of patients treated with chemoradiation showed that *XRCC1* Q399R = WW (versus PP or PW), *VEGF4032* CT/TT, and *TNF0629* = AA all confer a higher risk of severe RP. Patients carrying the *ATM* rs189037 variant AA genotype are at high risk of developing severe RP, particularly those who receive an MLD of  $\geq$ 19.0 Gy (17).

Incorporating SNPs into normal tissue complication probability predictive models such the Lyman dosimetric model has been shown to improve the model's ability to predict the likelihood of RP (24). Tucker *et al.* studied 16 potentially functional SNPs in genes related to DNA repair, cell cycling, TGF- $\beta$ 1, TNF $\alpha$ , folic acid metabolism, and angiogenesis from 143 patients. Five SNPs were selected for inclusion in a multivariate normal tissue complication probability model based on MLD alone. SNPs associated with an increased risk of severe RP were found in genes of TGF- $\beta$ 1, VEGF, TNF $\alpha$ , *XRCC1*, and *APEX1*. When smoking status was included in the multivariate model, SNPs associated with an increased risk of RP were found in TGF- $\beta$ 1, VEGF, and *XRCC3*. The SNP that most significantly improved the "t" of the Lyman model based on lung dosimetry alone was *XRCC*. These findings provide

evidence that SNPs may significantly improve the predictive ability of the Lyman model. Even with a small number of SNPs, it is possible to distinguish cohorts with >50% risk versus <10% risk of RP upon exposure to a high MLD.

However, independent validation of these findings has been challenging due to differences in endpoints, patient populations, and toxicity scoring criteria (27-30).

Large datasets with objective and consistent measurements of endpoints and tightly controlled variables are needed to validate these findings and to prospectively test the models developed in clinical settings. A significant drawback to identifying SNPs that are related to radiation injury is that large numbers of patients are needed, whereas the incidence of severe toxicity is fortunately low. Moreover, the major driver for radiation injury is the radiation dose and volume represented by the dose volume histogram (DVH), and this factor should always be taken into account when assessing markers of radiosensitivity.

### *Cytokine and proteomic markers*

TGF- $\beta$ 1 has been extensively studied as a biomarker predicting RILT (16,31-40). A persistently elevated plasma TGF- $\beta$ 1 above baseline level at the end of RT, the high ratio of TGF- $\beta$ 1 level over baseline during RT such as a significant elevation of TGF- $\beta$ 1 level at 4 weeks after RT are significantly associated with symptomatic RILT (35,36). A return of the plasma TGF- $\beta$ 1 to a normal level could identify patients who will not develop RP (31). However, TGF- $\beta$ 1 is also produced by lung cancer and also a known immune inhibitor, both of these may have confounded the toxicity results. TGF- $\beta$ 1 is also a sticky molecule, richly stored in platelet. Inconsistent TGF- $\beta$ 1 results were also noted, largely attributed to platelet contamination/degradation during blood sample handling procedures.

A complex network of cytokines may be involved in the process of RILT. Early variations in circulating interleukin (IL)-6 and IL-10 levels during RT were reported to be significantly associated with the risk of RP in a multivariate analysis ( $P=0.011$ ) (41,42). IL-6 levels before, during, and after thoracic radiation therapy also were reported to be significantly higher in those who developed pneumonitis (41). A low baseline level of IL-8 expression was consistently reported to be highly associated with RILT in two independent studies (42,43). However, most of these results have not been repeated by other investigators (44,45). Recently, it was proposed that the early response of cytokines within 1–24 hours after RT

may be a better predictor of RILT (46).

Proteomic approaches may identify new plasma protein markers for toxicity prediction. Cai *et al.* identified C4b-binding protein alpha chain, complement C3, and vitronectin as having significantly higher expression levels in patients with symptomatic RILT compared with patients without, based on both the datasets from the RT start to 3 months post-RT ( $P<0.01$ ) and from RT start to the end-RT ( $P<0.01$ ) (47). Using comparative, high-resolution liquid chromatography (LC)-MS, Oh *et al.* identified  $\alpha$ -2-macroglobulin ( $\alpha$ 2M) as the top candidate protein, validated independently using enzyme-linked immunosorbent assay (ELISA) on samples from an independent cohort of 20 patients ( $P=0.002$ ) (48). Unfortunately, effort and knowledge are limited on proteomics to identify and validate new biomarkers for planning of precision radiation, partially due to expensive cost associated with the testing.

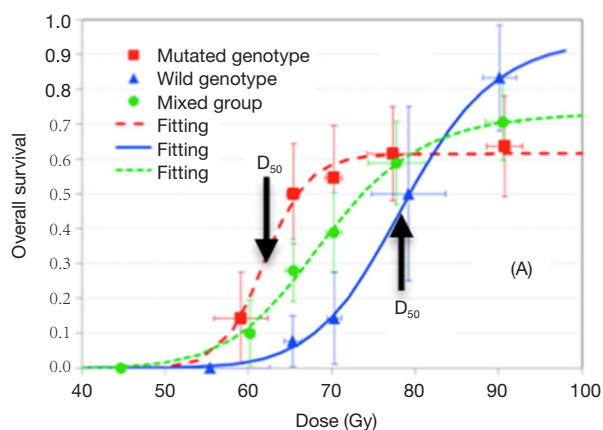
Blood biomarkers for toxicity can therefore help to personalize adaptive dose prescription (49).

### **Blood-based biomarkers for tumor control and survival**

Many factors contribute to tumor control and survival for a given patient. In addition to patient and tumor factors, treatment-related factors like radiation dose also play important roles. Further complicating this scenario, the factors related to normal tissue complications like immune suppression may also contribute to tumor control and survival because the immune system is also critical for tumor control.

### *Radiosensitivity biomarkers*

Tumor cells circulate in the blood such that these circulating tumor cells (CTCs) and their mutation types can be detected with advanced genomic technology in blood samples. CTCs may reflect the most active types of tumor cells in the primary tumor so that they may be a better representation of the tumor heterogeneity. While the mutation types have been used as radiosensitivity biomarkers for the tumor tissue samples, no systematic studies have been performed to correlate mutation types of CTCs with radiosensitivity because CTCs are a recently emerging field of study. Similarly, cell-free circulating tumor DNA (ctDNA) can be released from the tumor and quantified in blood. ctDNA analysis has become an established alternative to determine EGFR status (50). There is great



**Figure 1** Example dose-survival relationships for patients with sensitive and resistant genotypes. Genotypic candidate biomarkers were used to model the dose-response relationships in 92 patients with NSCLC treated with a wide range of radiation doses. D<sub>50</sub>, the dose for a 50% 2-year survival rate, differed remarkably according to the sensitivity of the individual patients (55). NSCLC, non-small cell lung cancer.

promise that serial ctDNA quantification during and after radiation therapy can be used to monitor tumor response to therapy (51-54). The quantification of early ctDNA release after radiation therapy, which reflects early cell death, is likely related to tumor radiosensitivity. Moreover, serial quantification of ctDNA might allow identification of certain mutations representing tumor subclones that do not respond favorably to radiation therapy, thus possibly reflecting the presence of radioresistant clones. Ongoing studies will clarify the potential of ctDNA quantification for radiosensitivity analysis.

It is challenging to study biomarkers for radiosensitivity, as heterogeneous responses from sensitive and resistant patients may cancel out the dose and survival relationship, and often there is not enough treatment variation to generate a dose-response relationship. In a study of 92 stage III NSCLC patients who received doses varying from 60–91 Gy, Jin *et al.* used a stratified dosimetric modeling approach to identify genotypic markers associated with DNA repair pathways for radiosensitivity assessment (55). These investigators reported that one SNP signature (combining ERCC2:rs238406 and ERCC1:rs11615) was significant for the radiotherapy dose-response. As shown in *Figure 1*, the RT doses required to generate a 50% OS rate at 2 years were 64 and 76 Gy, for sensitive and resistant groups, respectively. A future study with independent

validation is needed to confirm this finding.

### Non-radiosensitivity biomarkers

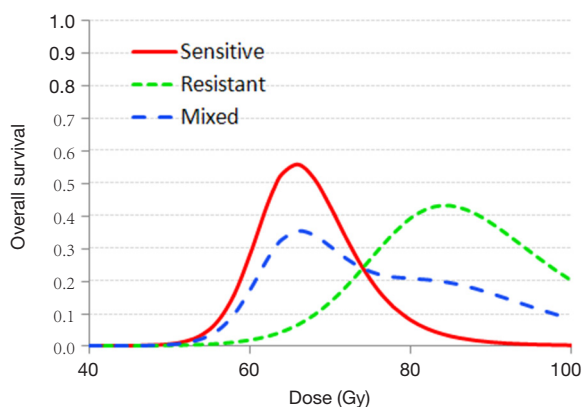
Many studies have reported that serum cytokine levels are correlated with survival (56). These include C-reactive protein, IL-6, IL-8, IL-10, and TNF- $\alpha$ . TGF- $\beta$ 1 expression at baseline and after treatment also was reported to correlate with disease status and treatment outcome in NSCLC patients (57). IL-6 and IL-8 were reported to significantly correlate with surgical treatment outcome in stage 1 NSCLC patients, and the combination of IL-6 and IL-8 increased the prediction value. Notably, the prediction value was better for stage IA patients than Stage IB patients.

While cytokines can be considered as immune markers, other proteins that directly reflect immune check points, such as programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), or the number of tumor-infiltrating CD8<sup>+</sup> T lymphocytes have been reported to be prognostic biomarkers for NSCLC. Most of the studies measured PD-L1 expression or CD8<sup>+</sup> T cells in tumor, while other studies measured them in the peripheral blood (58). A high expression level of PD-L1 was usually correlated with positive responses in PD-L1/PD-1-based immune checkpoint blockade treatments (59). However, the high PD-L1 expression level was reported to be a poor prognostic factor for patients treated with surgery (60,61). High indoleamine 2,3-dioxygenase (IDO) expression level also was reported to be associated with poor prognosis (62-64).

In NSCLC, associations between ctDNA, tumor size, stage, and the reduction after surgical resection or radiation therapy have been suggested (52,53,65). It remains to be determined under which circumstances are ctDNAs of prognostic value for NSCLC patients (51,54).

### Radiosensitivity biomarkers in combination of dosimetric factors to guide precision RT

Radiosensitivity biomarkers for both tumor and normal tissues are the key factors to determining the optimal radiation dose via a holistic approach. As the radiation dose to a tumor increases, the killing of tumor cells increases, and the tumor control/survival will increase. However, increasing the tumor dose also proportionally increases the doses delivered to the normal tissue, and damage to the immune system and other normal structures increases. This can reduce tumor control/survival. Therefore, an appropriate balance of these two competing factors will



**Figure 2** The relationship between dose, survival, and radiation sensitivity. The dose generating the best survival, i.e., the optimal dose, varies according to an individual's sensitivity, which was estimated by the genotype of ERCC1, a known DNA repair gene. The optimal doses were 66 Gy for sensitive patients and 84 Gy for resistant patients (55). ERCC, excision repair cross-complementing.

result in superior survival at an optimal prescription dose of treatment.

Figure 2 illustrates an example of how 2-year survival varies with the dose of radiation delivery to the tumor for patients with differing radiosensitivity. A patient with a radiation-sensitive genotype had an optimal prescription dose of 66 Gy, whereas a patient with radio-resistant genotypic features had an optimal dose of 85 Gy. Using this biomarker-based model, a personalized dose prescription may be generated to improve 2-year survival from 50% to 85% and 3% to 73% for hypothetically sensitive and resistant patients, respectively.

### Summary and future direction

Given the individualized complexity of tumors and patients, models that determine survival and toxicity of radiotherapy or any other treatment must take into account all spectrum variability as well as its dynamic state. However, until recently, there were no means to identify or quantify these complex biological processes. This has changed dramatically and with an ever-increasing pace. The genetic features of the tumor and the host, and increasingly also the epigenetic and proteomic characteristics, are being unraveled. However, radiation effects on the tumor and normal tissues are also affected by the dose and volume of irradiated

tissues. These dosimetric parameters are often reduced to a single figure such as the maximal or the mean dose or some volumetric-dose parameter  $V_x$  such as the volume of the lungs receiving 20 Gy or more.

A major challenge is the combination of all these biological, clinical, imaging, and dosimetric parameters in a validated prognostic and predictive model. Major improvements have been made, as summarized in the present review. It is important to stress that comprehensive dosimetric parameters must be taken into account in order to make the models complete, as dose and volume are still the major drivers of radiation effects in all tissues.

A combined biological-mathematical approach opens new doors beyond prognostication of patients; It allows truly precise oncological treatment. Indeed, the core for individualized and precision medicine is not only appropriate selection of patients, but even more the optimization of the therapeutic window on an individual basis. The holistic model will enable the determination of an individual dose-response relationship for each OAR for each tumor in each individual patient for the complete oncological treatment package. This includes, but is not limited to, radiotherapy alone. With an individual's dose-response curve in hand, it will be possible to test different doses of radiation and the combination of radiotherapy with any drug to plan treatments to achieve the optimal balance between toxicity and the chance for cure. Insights in the interactions between a multitude of parameters will lead to the discovery of new pathways and networks and fuel new biological research on target discovery for true precision medicine.

Very large datasets will sometimes, but not always, be required to construct these models, for the precision of the model is determined by the magnitude of the effects, which may or may not be large. Increasingly precise dosimetric models will reduce the number of patients needed to identify genetic determinants of toxicity, supporting the idea that these models can be established with a reasonable number of patients.

Therefore, we are moving away from intuitive changes in planning, such as lowering the dose for very radiosensitive patients without knowing the therapeutic window, to a thorough, objective and quantifiable holistic model.

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## Footnote

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