Towards Personalized Radiation Therapy of Liver Metastasis: Importance of Serial Blood Biomarkers

Ajdari, A.; Xie, Y.; Richter, C.; Duda, D. G.; Hong, T. S.; Bortfeld, T.;

Originally published:

March 2021

JCO Clinical Cancer Informatics 5(2021), 315-325

DOI: https://doi.org/10.1200/CCI.20.00118

Title:

Towards Personalized Radiation Therapy of Liver Metastasis: Importance of Serial Blood Biomarkers

Authors' list:

Ali Ajdari[1], Yunhe Xie[1], Christian Richter[2], Maximilian Niyazi[3], Dan Duda[1], Theodore S. Hong[1], Thomas Bortfeld[1]

Authors' affiliations:

[1] Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, NA

[2] OncoRay - National Center of Radiation Research in Oncology, Dresden, Germany

[3] Department of Radiation Oncology, Ludwig Maximilians University, Munich, Germany

Research funding: None

Corresponding Author:

Ali Ajdari, Ph.D.

Address: 100 Blossom Street

Boston MA, 02114

E-mail: aajdari@mgh.harvard.edu

Cell: (+1) (206) 604-5689

Running head: Importance of serial biomarkers in liver SBRT personalization

Part of the findings were previously reported in **2019 Annual Association of Physicists in Medicine (AAPM), San Antonio, TX, July 14 – 18**.

Disclaimer: None

Introduction

Today, more patient-specific data is routinely being collected throughout the radiation therapy (RT) course than ever before. From diagnosis to follow-up, patients go through several rounds of imaging, blood-draw, and physical examinations. The availability of serial (bio)marker data has the potential to uncover important physio-biological characteristics that were unknown at the beginning of the treatment, which in turn might help RT personalization. Such information can not only guide the treatment adaptation during RT, but can also facilitate timely and proactive response to complications after RT, well before the onset of the symptoms. Despite this, very limited attention has been given to systematically study the impact of such serial biomarker acquisition and how much *additional* predictive value they might contain. Instead, most researchers have adopted a rather “frozen” approach, in which treatment outcomes are solely estimated using (mostly) baseline predictors, disregarding the potentially invaluable information that might be collected during the RT course (1).

Liver is the most frequent site of cancer metastasis, with 60% of all primary tumors metastasizing to liver (2). Radiation therapy is a major treatment option in metastatic liver cancer, but potential liver damage remains a major dose-limiting factor. Therefore, finding subgroups of patients with higher or lower tolerance of radiation dose might help in identifying the favorable candidates for dose-escalation trials, while sparing the radiation damage for the more radiosensitive patients. This can be accomplished by personalized RT (3, 4).

In the case of liver metastasis RT, the literature on predictive biomarkers is relatively sparse. Due to their relatively inexpensive, minimally invasive, and repeatable acquisition, blood biomarkers are among the most studied biomarkers in liver cancer (5, 6). The systemic information provided by these biomarkers can potentially inform not only about local disease status, but also about the body’s overall response to treatment. Recently, we have reported the importance of baseline genotype information on predicting local failure in liver metastasis stereotactic body RT (SBRT) (7, 8). However, to what extent these and other potential (bio)markers of RT outcome might improve the prediction accuracy remains unknown.

We aim at addressing this gap by systematically investigating the predictive and prognostic potential of several classes of blood-based (bio)markers on liver metastasis RT response. We are particularly interested in quantifying the *informative* value of these biomarkers, both predictive and prognostic, and investigating whether they can improve upon the predictions made at baseline using traditional predictors of response such as tumor size, histology, and intra- and extra-hepatic disease burden. Specifically, our aim is to provide a proof-of-concept for personalized liver SBRT using baseline and mid-treatment biomarkers of radiation response.

Materials and Methods

Dataset

The clinical dataset is from a previously-reported phase II single arm clinical trial of liver metastasis patients (NCT01239381) (7). Eighty-nine adult (≥ 18 years old) patients with 1-4 hepatic metastatic lesions were included in the study. Median age was 68 years (range: 34-89) and 62.9% (n=56) were male. Most frequent primary tumor site was colorectal (n=34, 38%) cancer, followed by pancreatic (n=13, 15%) and esophagogastric cancers (n=12, 13%). Chemotherapy was the most common prior treatment received by the patients prior to RT initiation (n=75/85). All patients received passively scattered proton SBRT in 5 treatment fractions. Median RT dose, accounting for relative biological effectiveness of proton (RBE = 1.1), was 40 GyE (range = [30-50]). Other information is summarized in Table 1.

Study Endpoints

Given that the study population consisted of metastatic patients, RT response was measured using one local and one systemic RT endpoints: one-year local failure (LF) (which was the primary endpoint for the original prospective trial) was selected as the RT-specific endpoint and two-year overall survival (OS) was chosen as the systemic endpoint. Median follow-up time was 30.1 months (range = [14.7-53.8]), with one-year LF rate of 24.71% and two-year survival rate of 38%.

Baseline predictors of RT outcome

Dosimetric and clinicopathological markers studied were as follows:

- *Dosimetric*: mean liver dose (MLD), % of liver receiving over d Gy dose ($Vd, d = 5,10, \dots ,35$), and effective liver volume irradiated (V_{eff}).
- *Patient-specific*: Age, sex, and baseline liver function (cirrhosis status and CP score).
- *Disease-specific*: primary tumor site, number of hepatic metastasis, extent of prior therapy, and relative (% of total liver volume) and absolute size of total gross tumor volume (GTV).

Genetic markers

Additionally, the impact of *baseline* genotype information (GEN) on RT outcome was evaluated. We specifically focused on two oncogenes which were previously reported to be predictive of local failure in our current cohort (7). Baseline gene mutation data was available for 64 patients.

Serial biomarkers of RT outcome

Systemic markers: CBC

Three CBC-derived indices were studied as *global* biomarkers of tumor outcome (i.e., OS): absolute lymphocyte count (ALC), absolute neutrophil count (ANC), and platelets count (PLT). Additionally, two popular composite metrics were included in the analysis: neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). These inflammation indices have been often used as surrogates for immune system's response and associated with worse treatment outcome (9–13). CBC data was collected at baseline, $T0$ (n=88), and mid-treatment (before fraction 4, $T2$, n=80).

Inflammatory markers: IPC

Three candidate plasma cytokines collected from peripheral blood were analyzed at $T0$ and $T2$ as potential biomarkers of local response to SBRT: interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α. Both IL-6 and IL-8 are reportedly involved in hepatic cancer metastasis (14–16), as well as in inflammatory response to liver injury (17, 18).

TNF-α has also been associated with liver metastasis through TNF-α-induced protein (19). Fifty-five patients had IPC measurements at baseline and $T2$.

Predictive advantage of biomarkers

Assessing the predictive value of "new" biomarkers is a relatively established field in statistics and epidemiology (20–23). The gist is to fit a predictive model on the data with and without the new biomarker and assess the improvement in various goodness-of-fit, discrimination, or reclassification measures. To assess the predictive value of each biomarker class, first a baseline model was fitted using only the baseline predictors for each endpoint (i.e., clinicopathological and dosimetric factors). The predictive value of the new biomarkers was then assessed by adding them, one at a time, to the baseline model, fitting a new *biomarker-enhanced* predictive model over the data, and analyzing the impact on three main performance metrics: improvement in model fit was assessed by increase in likelihood ratio (LR) χ^2. A model with better discriminating power generally provides a greater variety of predictions, thus, increase in the predicted variance (PV) was selected as another metric of interest. Finally, change in discrimination power was assessed using area under the receiver-operating characteristic curve (AUC).

Statistical Analysis

All analyses were performed on the open-source statistical software R version 3.5.1 (R Project for Statistical Computing, Vienna, Austria). Univariate analysis was performed using Cox proportional hazard and log-rank test for time-to-event outcome and Fisher's exact test and Wilcoxon rank-sum test for binary outcomes. Logistic regression was used to build predictive models. A forward stepwise procedure was implemented to select the final model. At each step, the covariate with the highest decrease in χ^2 test p-value was added to the model and the procedure was repeated until no further improvement was achieved. To provide unbiased estimates of the performance metrics, bootstrapping (24) was performed with 1000 repetitions, as recommended by (25). Patients with missing data were removed from the analyses. Additionally, we removed all observations with more than 2 days of delay in their treatment to minimize the effect of timing of biomarker acquisition. Significance level was set at 0.05.

Results

Temporal change in biomarker expression

Between the baseline and the fourth fraction of SBRT, significant reduction was observed in both platelet count (223.8 37 th/mm^3 vs. 190.0 37 th/mm^3, p = 0.004) and absolute lymphocyte count (1.37 th/mm^3 vs. 1.01 37 th/mm^3, p = 0.0002), while plasma IL-6 levels increased from baseline to fraction 4 (2.83 vs. 3.35), however, this was not deemed significant (p > 0.05). With respect to RT effect, only the expression of plasma IL-6 and PLR were found to be significantly impacted by the delivered dose. On average, plasma IL-6 level increased from the baseline level with increasing liver volume irradiated above 5 Gy (V5). Patients with increased plasma IL-6 had received significantly lower V5 compared to those whose mid-treatment plasma IL-6 concertation decreased from baseline (33.43% vs. 46.78%, p = 0.01). Moreover, there was a significant association between pre-treatment IL-6 level and GTV size, with patients with larger tumor volumes (> median = 20.55 cc^3) showing higher plasma IL-6 level at baseline (3.88 vs. 1.74, p = 0.01). IL-6 level was not associated with any tumor histologies.

Univariate analysis

Predictors of local failure

None of the clinicopathological and dosimetric predictors were significantly associated with one-year risk of LF in the univariate analysis. As previously reported (26), mutation in *KRAS* gene was a strong predictor of LF (hazard ratio [HR] = 2.92 [95% CI = 1.17-7.28], p = 0.02). One-year LF rate was 69% vs. 31% in patients with and without *KRAS* mutation. We did not observe any association between *KRAS* mutation and primary tumor site (see Supplementary Table 5).

The most significant correlations among the IPC class were found between the plasma level of IL-6 at baseline (HR = 1.15 [1.05-1.26], p = 0.003) as well as at fraction 4 (HR = 1.07 [1.01-1.13], p = 0.01). Higher concentration of plasma IL-6, both at baseline and at T2 were associated with significantly worse local failure rate. Figure 1 shows the Kaplan-Meier curves for one-year local control. Note the loss of biomarker information (i.e., increase in p-value) upon discretization. Baseline and mid-treatment IL-6 kept their

significance even after accounting for GTV size (see Supplementary Table 7). Complete results are presented in the Supplementary Table 1-4 and Figure S2.

Predictors of overall survival

Among the clinicopathological factors, both GTV volume and its percentage of the whole liver volume were significant predictors of overall survival (HR = 1.003 and 1.01; p= 0.003 and 0.0005). V_{eff} was another significant predictor of OS, with an increase of 1% in V_{eff} leading to 1.7% increased likelihood of mortality at two years (HR = 1.017, p = 0.02). Additionally, patients with more intensive prior treatment (in terms of number of chemo lines administered as well as overall chemo length, in months) had significantly worse overall survival. Specifically, patients who had more than 3 lines of chemo and/or whose prior chemo treatment had taken over 6 months, had poorer prognosis (HR = 1.77 [1.05-3.03], p = 0.03; and HR = 1.84 [1.07-3.14], p = 0.03).

Among the CBC-derived biomarkers, baseline levels of PLR, NLR, and ALC were all significantly associated with OS (HR = 1.004,1.32, and 0.61; p = 0.004, < 0.0001, and 0.02). Patients with lower absolute lymphocyte count at baseline (≤ median = 1.1 th/mm^3) had significantly higher incidence of mortality following SBRT (2-year OS rate = 25% vs. 54%, p = 0.0002).

Predictive benefit of adding biomarkers

Table 2 summarizes the results of the analysis of added predictive value. The details of the final fitted models are given in the Supplementary Table 5. In predicting LF, addition of genotype information and baseline IPC information led to an increase of 0.06, and 0.07 in AUC compared to baseline model (using only clinicopathological and dosimetric factors). It also increased the variance of the predictions and improved model fit (LR χ^2). All these improvements were deemed statistically significant at α = 0.05.

Among the baseline biomarkers of OS, the addition of baseline CBC-derived biomarkers only marginally improved the model's predictive performance. Including mid-treatment CBC-derived biomarker information, however, led to significant gains in predictive performance across all metrics (p < 0.0001), increasing AUC from 0.72 in the baseline

model to 0.80. Figure 3, right panel, better illustrates these changes. Figure 4 shows the gain in AUC by adding GEN, IPC, and CBC class biomarkers.

Discussion

Identifying patients with higher/lower liver radiosensitivity is an important step towards personalization of liver RT, where outcome of treatment can be significantly improved by administering higher focal dose for those patients who are deemed “radioresistant”, while de-escalating the treatment course for more favorable responders. This crucially depends on the reliability of pre- and mid-treatment biomarkers for predicting the long and short-term RT response. A successful patient selection strategy can significantly help with designing personalized clinical trials and/or improving the treatment outcome. Our findings highlight the potential of several classes of blood biomarkers in identifying patients with higher/lower risk of poor cancer outcome, which goes beyond the traditional clinicopathological and dosimetric predictors.

Prior studies have reported the impact of larger tumor volume on increasing the risk of local failure after SBRT in liver metastasis patients (27, 28). In contrast, we did not find any significant association between tumor size and local failure, in line with the findings also reported in (29, 30). Colorectal primary tumors had been identified as predictors of more favorable local response after SBRT (31), an association our study failed to confirm, although this inconsistency might be due the sample size effect. No other clinical or dosimetric factors were statistically deemed significant as predictors of local failure, highlighting the challenge involved in predicting local response to SBRT using conventional predictors and emphasizing the need for finding and validation of novel biomarkers of local treatment response.

Our previous studies (7, 30) have demonstrated the significance of baseline genotype information on identifying a highly radioresistant subgroup of patients (i.e., those with mutations in both *KRAS* and *TP53* genes). Although genotype information can indeed be extremely valuable for patient selection, it is unlikely that it can be obtained for all patients, mainly due to its acquisition cost. Further, it has been reported that *KRAS*-

mutated tumors are extremely heterogenous (7, 32) and additional biomarkers are needed to find subgroups of patients with more homogenous treatment response.

Our analysis revealed baseline and mid-treatment plasma levels of IL-6 as other significant predictors of local failure after SBRT. Specifically, higher IL-6 levels at baseline and again at mid-treatment were associated with worse local control. The mid-treatment level of IL-6 seemed to possess significantly higher discriminative power (as judged by Figure 1) compared to the baseline level, which, given that plasma IL-6 increased with dose, seems to suggest that mid-treatment level of plasma IL-6 contain significant information about the effect of dose on treatment response, which is in line with previous reports regarding its RT-induced enhancement mediated through endothelial cells (33). Interestingly, plasma IL-6 levels increased with liver volume irradiated with lower dose (V5), but not with dose. Although this might be rather surprising, it's worth noting that the impact of low-dose bath (captured by V5-V10) on hepatic toxicity and change in a number of liver enzymes has recently been reported (34). Upon further analyses in our own dataset, we also found significant association between both V5 and mid-treatment IL-6 increase, and hepatic toxicity in terms of Child-Pugh score increase: specifically, we found that patients experiencing an increase in CP score on average had higher mid-treatment plasma IL-6 (1.95 vs. 7.06; p=0.0003). Together, these results might hint at a low dose-threshold for triggering IL-6 secretion, which in turn might imply that the change in plasma IL-6 might be detectable at even earlier fractions.

Unfortunately, despite the relatively high level of local control, overall survival rate in liver metastasis patients remain poor. Generally, physicians would like to avoid subjecting a patient with an extremely poor prognosis to the hardship of curative SBRT and the potential subsequent treatment-induced toxicity. As such, finding reliable prognostic biomarkers can be of great help when deciding on the best treatment option for each patient. Unlike prior studies which identified primary tumor histology as a significant prognostic factor for overall survival (27, 29), we did not find that to be the case in our cohort. Instead, our investigations showed that the information contained within regular CBC tests at baseline and during the SBRT course can be of help in this

regard. Specifically, we found that it is possible to stratify patients at baseline according to the baseline ALC, PLR and NLR levels (see Figure 2 and Supplementary Figure S1, left) obtained at baseline. Adding information on the change from baseline to fraction 4 further increased the inter-group discrimination (Supplementary Figure S1, right) and improved response prediction (Table 2). Given the important role played by these biomarkers in immunological processes (35–37), these reductions might reflect the baseline status and the change in the immune system response due to irradiation, which might have contributed to the poorer treatment outcome in patients with weaker immune profile. Recently, our group reported that in a subset of hypofractionated liver proton therapy (HPT) patients, lymphocyte depletion is predictive of overall survival, hinting at a possible immunomodulation effect from HPT in hepatocellular carcinoma patients.

Another important observation is the predictive advantage of mid-treatment biomarker measurements. In majority of the analysis, addition of mid-treatment biomarker information led to substantial improvement in predictive performance. Specifically, models containing mid-treatment information generally better fitted the data, had wider prediction variance (i.e., more subtle differences in patient risks could be detected using these compared to baseline models), and higher discriminatory power. Increasing the number of biomarkers can result in identification of higher number of patient subgroups, provided that the biomarkers be reliable and predictive (3). This is better illustrated in the Supplementary Figure S4, where addition of IL-6 at mid-treatment leads to the identification of an extremely radioresistant subgroup of *KRAS*-mutated patients (local failure rate = 100%, n = 3). Although the extremely small sample size prevents us from relying too much on these results, the reported synergistic role of *KRAS* oncogene and IL-6 mediated signaling in tumorigenesis and progression of pancreatic and lung cancers (38, 39) might suggest an interesting avenue for further research in this area.

As a proof of concept, in Figure 5, we illustrate an example for how combining baseline genotype information with mid-treatment plasma IL-6 measurement could result in identifying four distinct patient subgroups in terms of their predicted LF. In such a case, dose escalation might be worth considering for the most radioresistant group (patients

with *KRAS* mutated tumors and higher plasma IL-6 concentration after fraction 3), while the more radiosensitive patients (those with wildtype *KRAS* gene and lower mid-treatment plasma IL-6) might benefit from dose de-escalation. Of course, the realization of this hypothetical case is predicated on the validation of the results reported in our study in bigger and independent datasets. Ultimately, a combination of pre- and mid-treatment biomarkers, coupled with a rigorous mathematical framework for dynamic treatment plan adaptation are required for a truly personalized RT plan (1).

Despite the promising results, we should acknowledge the limitations of our study. First, independent validation on an external cohort is an important next step towards validating our findings. Towards this, we have arranged to collect necessary data for validation in an upcoming prospective clinical trial for liver metastasis SBRT patients. Second, we opted for a simple logistic regression as our predictive model, mainly due to its popularity in clinical research and ease of interpretation. It remains to be seen whether more sophisticated predictive models can improve these predictions; however, any potential improvement in predictive performance will likely come at the expense of the models' interpretability and/or generalizability. By design, the analysis of added predictive biomarker value is dependent on the choice of the underlying predictive model and the calibration of the baseline model (40, 41). Unfortunately, currently the field of liver metastasis SBRT lacks such well-calibrated models, especially for predicting treatment response. Consequently, a well-calibrated baseline model, if present, might make it harder for additional biomarkers to increase the predictive performance. Lastly, we acknowledge that due to the high number of factors analyzed and the limited sample size, the possibility of false positives due to multiple testing is increased. It is therefore important to take that into consideration while interpreting our findings.

Overall, our findings hint at few important observations: (i) potentially important predictive information might be contained within the routinely-collected and often-overlooked blood-based markers; (ii) even during the short course of SBRT (5 fractions), it is possible to (re)classify patients into responders and non-responders, beyond what is possible before the treatment, using information contained in mid-

treatment serum or plasma levels of blood samples; though, due to the relatively short course of SBRT, the turn-around time for laboratory analysis should be fast enough to allow for timely treatment adaptations based on such (re)classifications.

Conclusion

We investigated the role of serial serum and plasma blood-based biomarkers in predicting response to liver metastasis SBRT. The findings suggest that significant benefit in predicting RT response might be achieved by analyzing the information contained within certain immune system and inflammatory blood biomarkers. Additionally, the mid-treatment levels of a subset of these biomarkers contain significant predictive information not present in the baseline observations; thus, it might prove beneficial to continuously observe the change in the biomarker value in order to arrive at a more dynamic and accurate picture of the patient response, thereby paving the way towards a fully-personalized RT.

References

1. Ajdari A, Niyazi M, Nicolay NH, *et al.* Towards optimal stopping in radiation therapy. *Radiother. Oncol.* 2019;134:96–100.

2. Budczies J, von Winterfeld M, Klauschen F, *et al.* The landscape of metastatic progression patterns across major human cancers. *Oncotarget*. 2015;6:570–583.

3. Baumann M, Krause M, Overgaard J, *et al.* Radiation oncology in the era of precision medicine. *Nat. Rev. Cancer*. 2016;16:234–250.

4. Ree AH, Redalen KR. Personalized radiotherapy: Concepts, biomarkers and trial design. *Br. J. Radiol.* 2015;88.

5. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. *Cancer Discov.* 2014;4:650–61.

6. Xu-Welliver M, Carbone DP. Blood-based biomarkers in lung cancer: prognosis and treatment decisions. *Transl. Lung Cancer Res.* 2017;6:708–712.

7. Hong TS, Wo JY, Borger DR, *et al.* Phase II Study of Proton-Based Stereotactic Body Radiation Therapy for Liver Metastases: Importance of Tumor Genotype. *J. Natl. Cancer Inst.* 2017;109:1–8.

8. Wo JY, Zhu AX, McDonnell EI, *et al.* Clinical and Molecular Predictors of Local Failure After SBRT for Liver Metastases: A Secondary Analysis of a Prospective Phase II Trial. *Int. J. Radiat. Oncol.* 2015;93:S111–S112.

9. Haram A, Boland MR, Kelly ME, *et al.* The prognostic value of neutrophil-to-lymphocyte ratio in colorectal cancer: A systematic review. *J. Surg. Oncol.* 2017;115:470–479.

10. Li M-X, Liu X-M, Zhang X-F, *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: A systematic review and meta-analysis. *Int. J. Cancer*. 2014;134:2403–2413.

11. Li X, Chen ZH, Xing YF, *et al.* Platelet-to-lymphocyte ratio acts as a prognostic factor for patients with advanced hepatocellular carcinoma. *Tumor Biol.* 2015;36:2263–2269.

12. Choi WJ, Cleghorn MC, Jiang H, *et al.* Preoperative Neutrophil-to-Lymphocyte Ratio is a Better Prognostic Serum Biomarker than Platelet-to-Lymphocyte Ratio in Patients Undergoing Resection for Nonmetastatic Colorectal Cancer. *Ann. Surg. Oncol.* 2015;22:603–613.

13. Kwon HC, Kim SH, Oh SY, *et al.* Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers*. 2012;17:216–222.

14. Thomas H. IL-6 drives niche formation in pancreatic cancer liver metastasis. *Nat. Rev. Gastroenterol. Hepatol.* 2019;16:263.

15. Bai Z, Tai Y, Li W, *et al.* Gankyrin activates IL-8 to promote hepatic metastasis of colorectal cancer. *Cancer Res.* 2013;73:4548–4558.

16. Chen Y, Shi M, Yu GZ, *et al.* Interleukin-8, a promising predictor for prognosis of pancreatic cancer. *World J. Gastroenterol.* 2012;18:1123–1129.

17. Hartman ZC, Poage GM, Den Hollander P, *et al.* Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. *Cancer Res.* 2013;73:3470–3480.

18. Zimmermann HW, Seidler S, Gassler N, *et al.* Interleukin-8 is activated in patients with chronic liver diseases and associated with hepatic macrophage accumulation in human liver fibrosis. *PLoS One*. 2011;6.

19. Ham B, Fernandez MC, D'Costa Z, *et al.* The diverse roles of the TNF axis in cancer progression and metastasis. *Trends Cancer Res.* 2016;11:1–27.

20. Cook NR. Quantifying the added value of new biomarkers: how and how not. *Diagnostic Progn. Res.* 2018;2:14.

21. Pencina MJ, D'Agostino RB, Pencina KM, *et al.* Interpreting incremental value of markers added to risk prediction models. *Am. J. Epidemiol.* 2012;176:473–481.

22. Steyerberg EW, Pencina MJ, Lingsma HF, *et al.* Assessing the incremental value of diagnostic and prognostic markers: A review and illustration. *Eur. J. Clin. Invest.* 2012;42:216–228.

23. Pencina MJ, Parikh CR, Kimmel PL, *et al.* Statistical methods for building better biomarkers of chronic kidney disease. *Stat. Med.* 2019;38:1903–1917.

24. Efron B, Gong G. A Leisurely Look at the Bootstrap, the Jackknife, and Cross-Validation. *Am. Stat.* 1983;37:36.

25. Steyerberg EW, Harrell FE, Borsboom GJJM, *et al.* Internal validation of predictive models: Efficiency of some procedures for logistic regression analysis. *J. Clin. Epidemiol.* 2001;54:774–781.

26. Hong TS, Wo JY, Borger DR, *et al.* Phase II Study of Proton-Based Stereotactic Body Radiation Therapy for Liver Metastases: Importance of Tumor Genotype. *J. Natl. Cancer Inst.* 2017;109:1–8.

27. Mahadevan A, Blanck O, Lanciano R, *et al.* Stereotactic Body Radiotherapy (SBRT) for liver metastasis - clinical outcomes from the international multi-institutional RSSearch® Patient Registry. *Radiat. Oncol.* 2018;13.

28. Anstadt EJ, Shumway R, Colasanto J, *et al.* Single community-based institutional series of stereotactic body radiation therapy (SBRT) for treatment of liver metastases. *J. Gastrointest. Oncol.* 2019;10:330–338.

29. Scorsetti M, Comito T, Clerici E, *et al.* Phase II trial on SBRT for unresectable liver metastases: long-term outcome and prognostic factors of survival after 5 years of follow-up. *Radiat. Oncol.* 2018;13:234.

30. Wo JY, Zhu AX, McDonnell EI, *et al.* Clinical and Molecular Predictors of Local Failure After SBRT for Liver Metastases: A Secondary Analysis of a Prospective Phase II Trial. *Int. J. Radiat. Oncol.* 2015;93:S111–S112.

31. Merrell KW, Johnson JE, Mou B, *et al.* Stereotactic body radiotherapy for primary and metastatic liver tumors - the Mayo Clinic experience. *J. radiosurgery SBRT*. 2016;4:133–144.

32. Skoulidis F, Byers LA, Diao L, *et al.* Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov.* 2015;5:861–878.

33. Van Der Meeren A, Bertho JM, Vandamme M, *et al.* Ionizing radiation enhances IL-6 and IL-8 production by human endothelial cells. *Mediators Inflamm.* 1997;6:185–193.

34. Pursley J, El Naqa I, Sanford NN, *et al.* Dosimetric Analysis and Normal-Tissue Complication Probability Modeling of Child-Pugh Score and Albumin-Bilirubin Grade Increase After Hepatic Irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* 2020;107:986–995.

35. Ali RA, Wuescher LM, Worth RG. Platelets: Essential components of the immune system. *Curr. Trends Immunol.* 2015;16:65–78.

36. Cloutier N, Allaeys I, Marcoux G, *et al.* Platelets release pathogenic serotonin and return to circulation after immune complex-mediated sequestration. *Proc. Natl. Acad. Sci. U. S. A.* 2018;115:E1550–E1559.

37. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: Turning lymphocytes off. *Science (80-.).* 1998;280:243–248.

38. Zhang Y, Yan W, Collins MA, *et al.* Interleukin-6 is required for pancreatic cancer progression by promoting MAPK signaling activation and oxidative stress resistance. *Cancer Res.* 2013;73:6359–6374.

39. Brooks GD, McLeod L, Alhayyani S, *et al.* IL6 trans-signaling promotes KRAS-driven lung carcinogenesis. *Cancer Res.* 2016;76:866–876.

40. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: Issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat. Med.* 1996;15:361–387.

41. Harrell FE, Califf RM, Pryor DB, *et al.* Evaluating the Yield of Medical Tests. *JAMA J. Am. Med. Assoc.* 1982;247:2543–2546.

Title:

Towards Personalized Radiation Therapy of Liver Metastasis: Importance of Serial Blood Biomarkers

Authors' list:

Ali Ajdari[1], Yunhe Xie[1], Christian Richter[2], Maximilian Niyazi[3], Dan Duda[1], Theodore S. Hong[1], Thomas Bortfeld[1]

Authors' affiliations:

[1] Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, NA

[2] OncoRay - National Center of Radiation Research in Oncology, Dresden, Germany

[3] Department of Radiation Oncology, Ludwig Maximilians University, Munich, Germany

Research funding: None

Corresponding Author:

Ali Ajdari, Ph.D.

Address: 100 Blossom Street

Boston MA, 02114

E-mail: aajdari@mgh.harvard.edu

Cell: (+1) (206) 604-5689

Running head: Importance of serial biomarkers in liver SBRT personalization

Part of the findings were previously reported in **2019 Annual Association of Physicists in Medicine (AAPM), San Antonio, TX, July 14 – 18**.

Disclaimer: None

Introduction

Today, more patient-specific data is routinely being collected throughout the radiation therapy (RT) course than ever before. From diagnosis to follow-up, patients go through several rounds of imaging, blood-draw, and physical examinations. The availability of serial (bio)marker data has the potential to uncover important physio-biological characteristics that were unknown at the beginning of the treatment, which in turn might help RT personalization. Such information can not only guide the treatment adaptation during RT, but can also facilitate timely and proactive response to complications after RT, well before the onset of the symptoms. Despite this, very limited attention has been given to systematically study the impact of such serial biomarker acquisition and how much *additional* predictive value they might contain. Instead, most researchers have adopted a rather "frozen" approach, in which treatment outcomes are solely estimated using (mostly) baseline predictors, disregarding the potentially invaluable information that might be collected during the RT course (1).

Liver is the most frequent site of cancer metastasis, with 60% of all primary tumors metastasizing to liver (2). Radiation therapy is a major treatment option in metastatic liver cancer, but potential liver damage remains a major dose-limiting factor. Therefore, finding subgroups of patients with higher or lower tolerance of radiation dose might help in identifying the favorable candidates for dose-escalation trials, while sparing the radiation damage for the more radiosensitive patients. This can be accomplished by personalized RT (3, 4).

In the case of liver metastasis RT, the literature on predictive biomarkers is relatively sparse. Due to their relatively inexpensive, minimally invasive, and repeatable acquisition, blood biomarkers are among the most studied biomarkers in liver cancer (5, 6). The systemic information provided by these biomarkers can potentially inform not only about local disease status, but also about the body's overall response to treatment. Recently, we have reported the importance of baseline genotype information on predicting local failure in liver metastasis stereotactic body RT (SBRT) (7, 8). However, to what extent these and other potential (bio)markers of RT outcome might improve the prediction accuracy remains unknown.

We aim at addressing this gap by systematically investigating the predictive and prognostic potential of several classes of blood-based (bio)markers on liver metastasis RT response. We are particularly interested in quantifying the *informative* value of these biomarkers, both predictive and prognostic, and investigating whether they can improve upon the predictions made at baseline using traditional predictors of response such as tumor size, histology, and intra- and extra-hepatic disease burden. Specifically, our aim is to provide a proof-of-concept for personalized liver SBRT using baseline and mid-treatment biomarkers of radiation response.

Materials and Methods

Dataset

The clinical dataset is from a previously-reported phase II single arm clinical trial of liver metastasis patients (NCT01239381) (7). Eighty-nine adult (≥ 18 years old) patients with 1-4 hepatic metastatic lesions were included in the study. Median age was 68 years (range: 34-89) and 62.9% (n=56) were male. Most frequent primary tumor site was colorectal (n=34, 38%) cancer, followed by pancreatic (n=13, 15%) and esophagogastric cancers (n=12, 13%). Chemotherapy was the most common prior treatment received by the patients prior to RT initiation (n=75/85). All patients received passively scattered proton SBRT in 5 treatment fractions. Median RT dose, accounting for relative biological effectiveness of proton (RBE = 1.1), was 40 GyE (range = [30-50]). Other information is summarized in Table 1.

Formatted: Highlight

Formatted: Highlight

Study Endpoints

Given that the study population consisted of metastatic patients, RT response was measured using one local and one systemic RT endpoints: one-year local failure (LF) (which was the primary endpoint for the original prospective trial) was selected as the RT-specific endpoint and two-year overall survival (OS) was chosen as the systemic endpoint. Median follow-up time was 30.1 months (range = [14.7-53.8]), with one-year LF rate of 24.71% and two-year survival rate of 38%.

Baseline predictors of RT outcome

Dosimetric and clinicopathological markers studied were as follows:

- *Dosimetric*: mean liver dose (MLD), % of liver receiving over d Gy dose ($Vd, d = 5,10, \dots ,35$), and effective liver volume irradiated (V_{eff}).
- *Patient-specific*: Age, sex, and baseline liver function (cirrhosis status and CP score).
- *Disease-specific*: primary tumor site, number of hepatic metastasis, extent of prior therapy, and relative (% of total liver volume) and absolute size of total gross tumor volume (GTV).

Genetic markers

Additionally, the impact of *baseline* genotype information (GEN) on RT outcome was evaluated. We specifically focused on two oncogenes which were previously reported to be predictive of local failure in our current cohort (7). Baseline gene mutation data was available for 64 patients.

Serial biomarkers of RT outcome

Systemic markers: CBC

Three CBC-derived indices were studied as *global* biomarkers of tumor outcome (i.e., OS): absolute lymphocyte count (ALC), absolute neutrophil count (ANC), and platelets count (PLT). Additionally, two popular composite metrics were included in the analysis: neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). These inflammation indices have been often used as surrogates for immune system's response and associated with worse treatment outcome (9–13). CBC data was collected at baseline, $T0$ (n=88), and mid-treatment (before fraction 4, $T2$, n=80).

Inflammatory markers: IPC

Three candidate plasma cytokines collected from peripheral blood were analyzed at $T0$ and $T2$ as potential biomarkers of local response to SBRT: interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α. Both IL-6 and IL-8 are reportedly involved in hepatic cancer metastasis (14–16), as well as in inflammatory response to liver injury (17, 18).

TNF-α has also been associated with liver metastasis through TNF-α-induced protein (19). Fifty-five patients had IPC measurements at baseline and $T2$.

Predictive advantage of biomarkers

Assessing the predictive value of "new" biomarkers is a relatively established field in statistics and epidemiology (20–23). The gist is to fit a predictive model on the data with and without the new biomarker and assess the improvement in various goodness-of-fit, discrimination, or reclassification measures. To assess the predictive value of each biomarker class, first a baseline model was fitted using only the baseline predictors for each endpoint (i.e., clinicopathological and dosimetric factors). The predictive value of the new biomarkers was then assessed by adding them, one at a time, to the baseline model, fitting a new *biomarker-enhanced* predictive model over the data, and analyzing the impact on three main performance metrics: improvement in model fit was assessed by increase in likelihood ratio (LR) χ^2. A model with better discriminating power generally provides a greater variety of predictions, thus, increase in the predicted variance (PV) was selected as another metric of interest. Finally, change in discrimination power was assessed using area under the receiver-operating characteristic curve (AUC).

Statistical Analysis

All analyses were performed on the open-source statistical software R version 3.5.1 (R Project for Statistical Computing, Vienna, Austria). Univariate analysis was performed using Cox proportional hazard and log-rank test for time-to-event outcome and Fisher's exact test and Wilcoxon rank-sum test for binary outcomes. Logistic regression was used to build predictive models. A forward stepwise procedure was implemented to select the final model. At each step, the covariate with the highest decrease in χ^2 test p-value was added to the model and the procedure was repeated until no further improvement was achieved. To provide unbiased estimates of the performance metrics, bootstrapping (24) was performed with 1000 repetitions, as recommended by (25). Patients with missing data were removed from the analyses. Additionally, we removed all observations with more than 2 days of delay in their treatment to minimize the effect of timing of biomarker acquisition. Significance level was set at 0.05.

Results

Temporal change in biomarker expression

Between the baseline and the fourth fraction of SBRT, significant reduction was observed in both platelet count (223.8 37 th/mm^3 vs. 190.0 37 th/mm^3, p = 0.004) and absolute lymphocyte count (1.37 th/mm^3 vs. 1.01 37 th/mm^3, p = 0.0002), while plasma IL-6 levels increased from baseline to fraction 4 (2.83 vs. 3.35), however, this was not deemed significant (p > 0.05). With respect to RT effect, only the expression of plasma IL-6 and PLR were found to be significantly impacted by the delivered dose. On average, plasma IL-6 level increased from the baseline level with increasing liver volume irradiated above 5 Gy (V5). Patients with increased plasma IL-6 had received significantly lower V5 compared to those whose mid-treatment plasma IL-6 concertation decreased from baseline (33.43% vs. 46.78%, p = 0.01). Moreover, there was a significant association between pre-treatment IL-6 level and GTV size, with patients with larger tumor volumes (> median = 20.55 cc^3) showing higher plasma IL-6 level at baseline (3.88 vs. 1.74, p = 0.01). IL-6 level was not associated with any tumor histologies.

Univariate analysis

Predictors of local failure

None of the clinicopathological and dosimetric predictors were significantly associated with one-year risk of LF in the univariate analysis. As previously reported (26), mutation in *KRAS* gene was a strong predictor of LF (hazard ratio [HR] = 2.92 [95% CI = 1.17-7.28], p = 0.02). One-year LF rate was 69% vs. 31% in patients with and without *KRAS* mutation. We did not observe any association between *KRAS* mutation and primary tumor site (see Supplementary Table 5).

The most significant correlations among the IPC class were found between the plasma level of IL-6 at baseline (HR = 1.15 [1.05-1.26], p = 0.003) as well as at fraction 4 (HR = 1.07 [1.01-1.13], p = 0.01). Higher concentration of plasma IL-6, both at baseline and at T2 were associated with significantly worse local failure rate. Figure 1 shows the Kaplan-Meier curves for one-year local control. Note the loss of biomarker information (i.e., increase in p-value) upon discretization. Baseline and mid-treatment IL-6 kept their

significance even after accounting for GTV size (see Supplementary Table 7). Complete results are presented in the Supplementary Table 1-4 and Figure S2.

Predictors of overall survival

Among the clinicopathological factors, both GTV volume and its percentage of the whole liver volume were significant predictors of overall survival (HR = 1.003 and 1.01; p= 0.003 and 0.0005). V_{eff} was another significant predictor of OS, with an increase of 1% in V_{eff} leading to 1.7% increased likelihood of mortality at two years (HR = 1.017, p = 0.02). Additionally, patients with more intensive prior treatment (in terms of number of chemo lines administered as well as overall chemo length, in months) had significantly worse overall survival. Specifically, patients who had more than 3 lines of chemo and/or whose prior chemo treatment had taken over 6 months, had poorer prognosis (HR = 1.77 [1.05-3.03], p = 0.03; and HR = 1.84 [1.07-3.14], p = 0.03).

Among the CBC-derived biomarkers, baseline levels of PLR, NLR, and ALC were all significantly associated with OS (HR = 1.004,1.32, and 0.61; p = 0.004, < 0.0001, and 0.02). Patients with lower absolute lymphocyte count at baseline (≤ median = 1.1 th/mm^3) had significantly higher incidence of mortality following SBRT (2-year OS rate = 25% vs. 54%, p = 0.0002).

Predictive benefit of adding biomarkers

Table 2~~1~~ summarizes the results of the analysis of added predictive value. The details of the final fitted models are given in the Supplementary Table 5. In predicting LF, addition of genotype information and baseline IPC information led to an increase of 0.06, and 0.07 in AUC compared to baseline model (using only clinicopathological and dosimetric factors). It also increased the variance of the predictions and improved model fit (LR χ^2). All these improvements were deemed statistically significant at α = 0.05.

Among the baseline biomarkers of OS, the addition of baseline CBC-derived biomarkers only marginally improved the model's predictive performance. Including mid-treatment CBC-derived biomarker information, however, led to significant gains in predictive performance across all metrics (p < 0.0001), increasing AUC from 0.72 in the baseline

model to 0.80. Figure 3, right panel, better illustrates these changes. Figure 4 shows the gain in AUC by adding GEN, IPC, and CBC class biomarkers.

Discussion

Identifying patients with higher/lower liver radiosensitivity is an important step towards personalization of liver RT, where outcome of treatment can be significantly improved by administering higher focal dose for those patients who are deemed “radioresistant”, while de-escalating the treatment course for more favorable responders. This crucially depends on the reliability of pre- and mid-treatment biomarkers for predicting the long and short-term RT response. A successful patient selection strategy can significantly help with designing personalized clinical trials and/or improving the treatment outcome. Our findings highlight the potential of several classes of blood biomarkers in identifying patients with higher/lower risk of poor cancer outcome, which goes beyond the traditional clinicopathological and dosimetric predictors.

Prior studies have reported the impact of larger tumor volume on increasing the risk of local failure after SBRT in liver metastasis patients (27, 28). In contrast, we did not find any significant association between tumor size and local failure, in line with the findings also reported in (29, 30). Colorectal primary tumors had been identified as predictors of more favorable local response after SBRT (31), an association our study failed to confirm, although this inconsistency might be due the sample size effect. No other clinical or dosimetric factors were statistically deemed significant as predictors of local failure, highlighting the challenge involved in predicting local response to SBRT using conventional predictors and emphasizing the need for finding and validation of novel biomarkers of local treatment response.

Our previous studies (7, 30) have demonstrated the significance of baseline genotype information on identifying a highly radioresistant subgroup of patients (i.e., those with mutations in both *KRAS* and *TP53* genes). Although genotype information can indeed be extremely valuable for patient selection, it is unlikely that it can be obtained for all patients, mainly due to its acquisition cost. Further, it has been reported that *KRAS*-

Formatted: Highlight

mutated tumors are extremely heterogenous (7, 32) and additional biomarkers are needed to find subgroups of patients with more homogenous treatment response.

Our analysis revealed baseline and mid-treatment plasma levels of IL-6 as other significant predictors of local failure after SBRT. Specifically, higher IL-6 levels at baseline and again at mid-treatment were associated with worse local control. The mid-treatment level of IL-6 seemed to possess significantly higher discriminative power (as judged by Figure 1) compared to the baseline level, which, given that plasma IL-6 increased with dose, seems to suggest that mid-treatment level of plasma IL-6 contain significant information about the effect of dose on treatment response, which is in line with previous reports regarding its RT-induced enhancement mediated through endothelial cells (33). Interestingly, plasma IL-6 levels increased with liver volume irradiated with lower dose (V5), but not with dose. Although this might be rather surprising, it's worth noting that the impact of low-dose bath (captured by V5-V10) on hepatic toxicity and change in a number of liver enzymes has recently been reported (34). Upon further analyses in our own dataset, we also found significant association between both V5 and mid-treatment IL-6 increase, and hepatic toxicity in terms of Child-Pugh score increase: specifically, we found that patients experiencing an increase in CP score on average had higher mid-treatment plasma IL-6 (1.95 vs. 7.06; p=0.0003). Together, these results might hint at a low dose-threshold for triggering IL-6 secretion, which in turn might imply that the change in plasma IL-6 might be detectable at even earlier fractions.

Unfortunately, despite the relatively high level of local control, overall survival rate in liver metastasis patients remain poor. Generally, physicians would like to avoid subjecting a patient with an extremely poor prognosis to the hardship of curative SBRT and the potential subsequent treatment-induced toxicity. As such, finding reliable prognostic biomarkers can be of great help when deciding on the best treatment option for each patient. Unlike prior studies which identified primary tumor histology as a significant prognostic factor for overall survival (27, 29), we did not find that to be the case in our cohort. Instead, our investigations showed that the information contained within regular CBC tests at baseline and during the SBRT course can be of help in this

regard. Specifically, we found that it is possible to stratify patients at baseline according to the baseline ALC, PLR and NLR levels (see Figure 2 and Supplementary Figure S1, left) obtained at baseline. Adding information on the change from baseline to fraction 4 further increased the inter-group discrimination (Supplementary Figure S1, right) and improved response prediction (Table 2~~1~~). Given the important role played by these biomarkers in immunological processes (35–37), these reductions might reflect the baseline status and the change in the immune system response due to irradiation, which might have contributed to the poorer treatment outcome in patients with weaker immune profile. Recently, our group reported that in a subset of hypofractionated liver proton therapy (HPT) patients, lymphocyte depletion is predictive of overall survival, hinting at a possible immunomodulation effect from HPT in hepatocellular carcinoma patients.

Another important observation is the predictive advantage of mid-treatment biomarker measurements. In majority of the analysis, addition of mid-treatment biomarker information led to substantial improvement in predictive performance. Specifically, models containing mid-treatment information generally better fitted the data, had wider prediction variance (i.e., more subtle differences in patient risks could be detected using these compared to baseline models), and higher discriminatory power. Increasing the number of biomarkers can result in identification of higher number of patient subgroups, provided that the biomarkers be reliable and predictive (3). This is better illustrated in the Supplementary Figure S4, where addition of IL-6 at mid-treatment leads to the identification of an extremely radioresistant subgroup of *KRAS*-mutated patients (local failure rate = 100%, n = 3). Although the extremely small sample size prevents us from relying too much on these results, the reported synergistic role of *KRAS* oncogene and IL-6 mediated signaling in tumorigenesis and progression of pancreatic and lung cancers (38, 39) might suggest an interesting avenue for further research in this area.

As a proof of concept, in Figure 5, we illustrate an example for how combining baseline genotype information with mid-treatment plasma IL-6 measurement could result in identifying four distinct patient subgroups in terms of their predicted LF. In such a case, dose escalation might be worth considering for the most radioresistant group (patients

with *KRAS* mutated tumors and higher plasma IL-6 concentration after fraction 3), while the more radiosensitive patients (those with wildtype *KRAS* gene and lower mid-treatment plasma IL-6) might benefit from dose de-escalation. Of course, the realization of this hypothetical case is predicated on the validation of the results reported in our study in bigger and independent datasets. Ultimately, a combination of pre- and mid-treatment biomarkers, coupled with a rigorous mathematical framework for dynamic treatment plan adaptation are required for a truly personalized RT plan (1).

Despite the promising results, we should acknowledge the limitations of our study. First, independent validation on an external cohort is an important next step towards validating our findings. Towards this, we have arranged to collect necessary data for validation in an upcoming prospective clinical trial for liver metastasis SBRT patients. Second, we opted for a simple logistic regression as our predictive model, mainly due to its popularity in clinical research and ease of interpretation. It remains to be seen whether more sophisticated predictive models can improve these predictions; however, any potential improvement in predictive performance will likely come at the expense of the models' interpretability and/or generalizability. By design, the analysis of added predictive biomarker value is dependent on the choice of the underlying predictive model and the calibration of the baseline model (40, 41). Unfortunately, currently the field of liver metastasis SBRT lacks such well-calibrated models, especially for predicting treatment response. Consequently, a well-calibrated baseline model, if present, might make it harder for additional biomarkers to increase the predictive performance. Lastly, we acknowledge that due to the high number of factors analyzed and the limited sample size, the possibility of false positives due to multiple testing is increased. It is therefore important to take that into consideration while interpreting our findings.

Overall, our findings hint at few important observations: (i) potentially important predictive information might be contained within the routinely-collected and often-overlooked blood-based markers; (ii) even during the short course of SBRT (5 fractions), it is possible to (re)classify patients into responders and non-responders, beyond what is possible before the treatment, using information contained in mid-

treatment serum or plasma levels of blood samples; though, due to the relatively short course of SBRT, the turn-around time for laboratory analysis should be fast enough to allow for timely treatment adaptations based on such (re)classifications.

Conclusion

We investigated the role of serial serum and plasma blood-based biomarkers in predicting response to liver metastasis SBRT. The findings suggest that significant benefit in predicting RT response might be achieved by analyzing the information contained within certain immune system and inflammatory blood biomarkers. Additionally, the mid-treatment levels of a subset of these biomarkers contain significant predictive information not present in the baseline observations; thus, it might prove beneficial to continuously observe the change in the biomarker value in order to arrive at a more dynamic and accurate picture of the patient response, thereby paving the way towards a fully-personalized RT.

References

1. Ajdari A, Niyazi M, Nicolay NH, *et al.* Towards optimal stopping in radiation therapy. *Radiother. Oncol.* 2019;134:96–100.

2. Budczies J, von Winterfeld M, Klauschen F, *et al.* The landscape of metastatic progression patterns across major human cancers. *Oncotarget*. 2015;6:570–583.

3. Baumann M, Krause M, Overgaard J, *et al.* Radiation oncology in the era of precision medicine. *Nat. Rev. Cancer*. 2016;16:234–250.

4. Ree AH, Redalen KR. Personalized radiotherapy: Concepts, biomarkers and trial design. *Br. J. Radiol.* 2015;88.

5. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. *Cancer Discov.* 2014;4:650–61.

6. Xu-Welliver M, Carbone DP. Blood-based biomarkers in lung cancer: prognosis and treatment decisions. *Transl. Lung Cancer Res.* 2017;6:708–712.

7. Hong TS, Wo JY, Borger DR, *et al.* Phase II Study of Proton-Based Stereotactic Body Radiation Therapy for Liver Metastases: Importance of Tumor Genotype. *J. Natl. Cancer Inst.* 2017;109:1–8.

8. Wo JY, Zhu AX, McDonnell EI, *et al.* Clinical and Molecular Predictors of Local Failure After SBRT for Liver Metastases: A Secondary Analysis of a Prospective Phase II Trial. *Int. J. Radiat. Oncol.* 2015;93:S111–S112.

9. Haram A, Boland MR, Kelly ME, *et al.* The prognostic value of neutrophil-to-lymphocyte ratio in colorectal cancer: A systematic review. *J. Surg. Oncol.* 2017;115:470–479.

10. Li M-X, Liu X-M, Zhang X-F, *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: A systematic review and meta-analysis. *Int. J. Cancer*. 2014;134:2403–2413.

11. Li X, Chen ZH, Xing YF, *et al.* Platelet-to-lymphocyte ratio acts as a prognostic factor for patients with advanced hepatocellular carcinoma. *Tumor Biol.* 2015;36:2263–2269.

12. Choi WJ, Cleghorn MC, Jiang H, *et al.* Preoperative Neutrophil-to-Lymphocyte Ratio is a Better Prognostic Serum Biomarker than Platelet-to-Lymphocyte Ratio in Patients Undergoing Resection for Nonmetastatic Colorectal Cancer. *Ann. Surg. Oncol.* 2015;22:603–613.

13. Kwon HC, Kim SH, Oh SY, *et al.* Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers*. 2012;17:216–222.

14. Thomas H. IL-6 drives niche formation in pancreatic cancer liver metastasis. *Nat. Rev. Gastroenterol. Hepatol.* 2019;16:263.

15. Bai Z, Tai Y, Li W, *et al.* Gankyrin activates IL-8 to promote hepatic metastasis of colorectal cancer. *Cancer Res.* 2013;73:4548–4558.

16. Chen Y, Shi M, Yu GZ, *et al.* Interleukin-8, a promising predictor for prognosis of pancreatic cancer. *World J. Gastroenterol.* 2012;18:1123–1129.

17. Hartman ZC, Poage GM, Den Hollander P, *et al.* Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. *Cancer Res.* 2013;73:3470–3480.

18. Zimmermann HW, Seidler S, Gassler N, *et al.* Interleukin-8 is activated in patients with chronic liver diseases and associated with hepatic macrophage accumulation in human liver fibrosis. *PLoS One*. 2011;6.

19. Ham B, Fernandez MC, D'Costa Z, *et al.* The diverse roles of the TNF axis in cancer progression and metastasis. *Trends Cancer Res.* 2016;11:1–27.

20. Cook NR. Quantifying the added value of new biomarkers: how and how not. *Diagnostic Progn. Res.* 2018;2:14.

21. Pencina MJ, D'Agostino RB, Pencina KM, *et al.* Interpreting incremental value of markers added to risk prediction models. *Am. J. Epidemiol.* 2012;176:473–481.

22. Steyerberg EW, Pencina MJ, Lingsma HF, *et al.* Assessing the incremental value of diagnostic and prognostic markers: A review and illustration. *Eur. J. Clin. Invest.* 2012;42:216–228.

23. Pencina MJ, Parikh CR, Kimmel PL, *et al.* Statistical methods for building better biomarkers of chronic kidney disease. *Stat. Med.* 2019;38:1903–1917.

24. Efron B, Gong G. A Leisurely Look at the Bootstrap, the Jackknife, and Cross-Validation. *Am. Stat.* 1983;37:36.

25. Steyerberg EW, Harrell FE, Borsboom GJJM, *et al.* Internal validation of predictive models: Efficiency of some procedures for logistic regression analysis. *J. Clin. Epidemiol.* 2001;54:774–781.

26. Hong TS, Wo JY, Borger DR, *et al.* Phase II Study of Proton-Based Stereotactic Body Radiation Therapy for Liver Metastases: Importance of Tumor Genotype. *J. Natl. Cancer Inst.* 2017;109:1–8.

27. Mahadevan A, Blanck O, Lanciano R, *et al.* Stereotactic Body Radiotherapy (SBRT) for liver metastasis - clinical outcomes from the international multi-institutional RSSearch® Patient Registry. *Radiat. Oncol.* 2018;13.

28. Anstadt EJ, Shumway R, Colasanto J, *et al.* Single community-based institutional series of stereotactic body radiation therapy (SBRT) for treatment of liver metastases. *J. Gastrointest. Oncol.* 2019;10:330–338.

29. Scorsetti M, Comito T, Clerici E, *et al.* Phase II trial on SBRT for unresectable liver metastases: long-term outcome and prognostic factors of survival after 5 years of follow-up. *Radiat. Oncol.* 2018;13:234.

30. Wo JY, Zhu AX, McDonnell EI, *et al.* Clinical and Molecular Predictors of Local Failure After SBRT for Liver Metastases: A Secondary Analysis of a Prospective Phase II Trial. *Int. J. Radiat. Oncol.* 2015;93:S111–S112.

31. Merrell KW, Johnson JE, Mou B, *et al.* Stereotactic body radiotherapy for primary and metastatic liver tumors - the Mayo Clinic experience. *J. radiosurgery SBRT*. 2016;4:133–144.

32. Skoulidis F, Byers LA, Diao L, *et al.* Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov.* 2015;5:861–878.

33. Van Der Meeren A, Bertho JM, Vandamme M, *et al.* Ionizing radiation enhances IL-6 and IL-8 production by human endothelial cells. *Mediators Inflamm.* 1997;6:185–193.

34. Pursley J, El Naqa I, Sanford NN, *et al.* Dosimetric Analysis and Normal-Tissue Complication Probability Modeling of Child-Pugh Score and Albumin-Bilirubin Grade Increase After Hepatic Irradiation. *Int. J. Radiat. Oncol. Biol. Phys.* 2020;107:986–995.

35. Ali RA, Wuescher LM, Worth RG. Platelets: Essential components of the immune system. *Curr. Trends Immunol.* 2015;16:65–78.

36. Cloutier N, Allaeys I, Marcoux G, *et al.* Platelets release pathogenic serotonin and return to circulation after immune complex-mediated sequestration. *Proc. Natl. Acad. Sci. U. S. A.* 2018;115:E1550–E1559.

37. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: Turning lymphocytes off. *Science (80-.).* 1998;280:243–248.

38. Zhang Y, Yan W, Collins MA, *et al.* Interleukin-6 is required for pancreatic cancer progression by promoting MAPK signaling activation and oxidative stress resistance. *Cancer Res.* 2013;73:6359–6374.

39. Brooks GD, McLeod L, Alhayyani S, *et al.* IL6 trans-signaling promotes KRAS-driven lung carcinogenesis. *Cancer Res.* 2016;76:866–876.

40. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: Issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat. Med.* 1996;15:361–387.

41. Harrell FE, Califf RM, Pryor DB, *et al.* Evaluating the Yield of Medical Tests. *JAMA J. Am. Med. Assoc.* 1982;247:2543–2546.

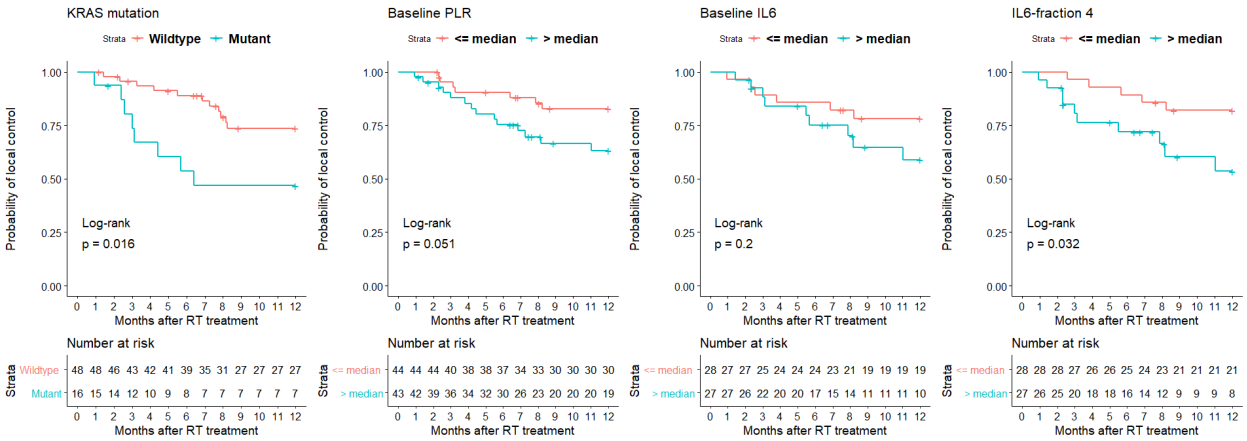

Figure 1. Kaplan-Meier curves for the 1-year local control rate among the study population, stratified according to the expression of blood biomarkers. Statistical tests were performed using log-rank test. PLR = platelets-to-lymphocyte ratio; IL6 = plasma interleukin 6; RT = radiation therapy. Mid-treatment plasma IL-6 level was measured before fraction 4 of stereotactic body RT. Median values: PLR=175.71, baseline IL6 = 1.61 pg/ml, IL6-fraction 4 = 1.52 pg/ml.

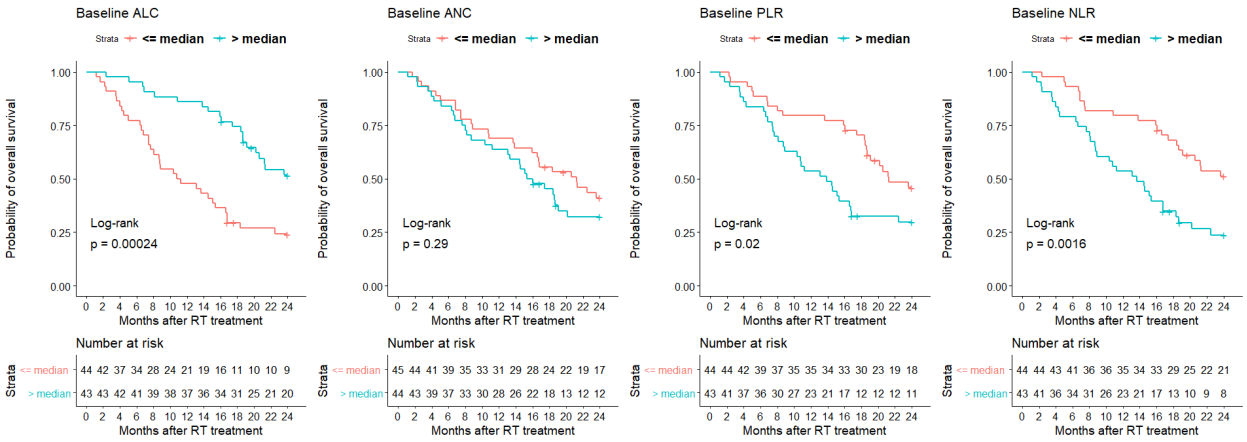

Figure 2. Kaplan-Meier curves for 2-year overall survival, stratified by the expression of CBC-derived biomarkers. P-values using log-rank test for patient groups stratified over median value of the biomarker. CBC = complete blood count; ALC=absolute lymphocyte count; ANC = absolute neutrophil count; PLR=platelets-to-lymphocyte ratio; NLR=Neutrophil-to-lymphocyte ratio; RT = radiation therapy. Median values: Baseline ALC = 1.1 th/mm^3, baseline ANC = 4.48 th/mm^3, baseline PLR = 175.71, baseline NLR = 3.89.

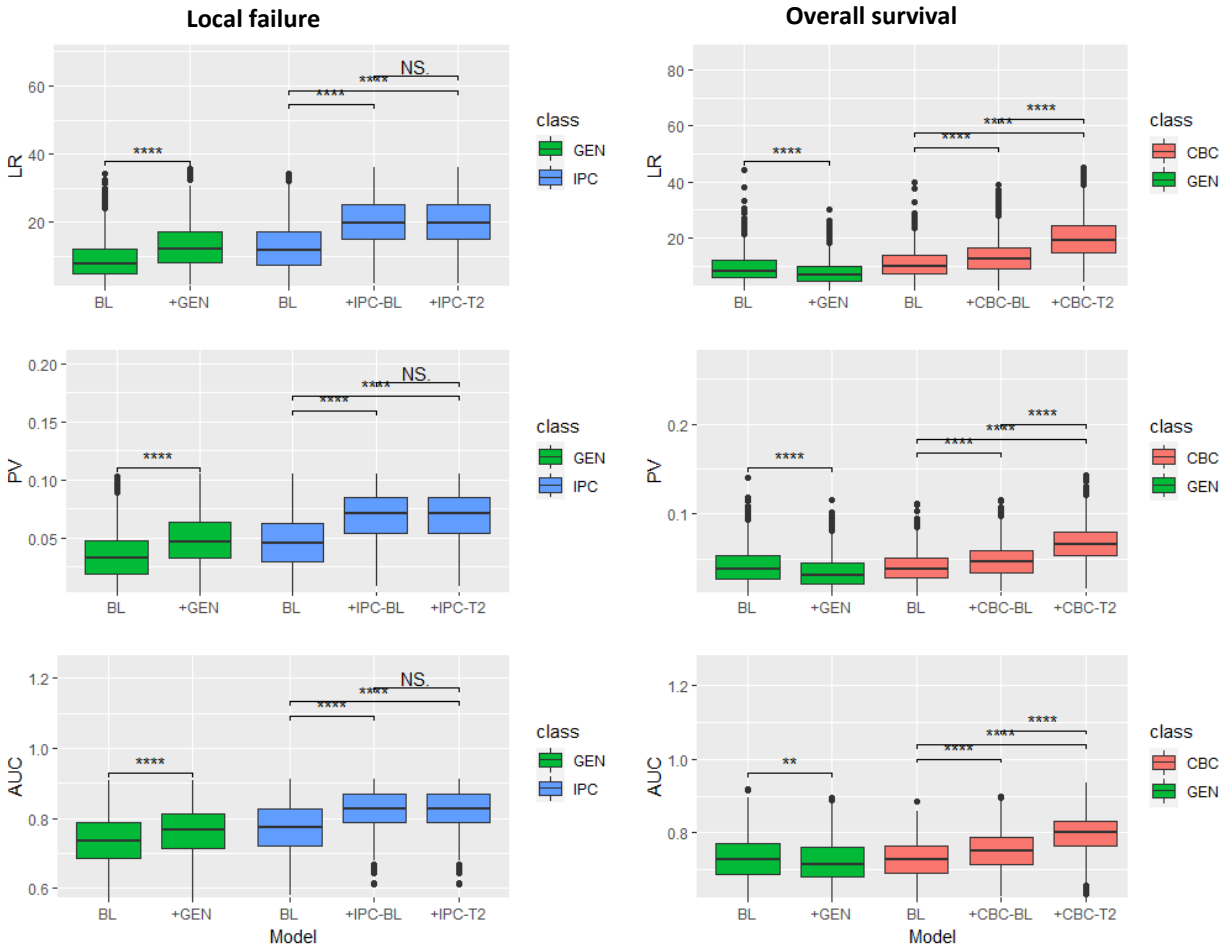

*Figure 3. Bootstrapped predictive performance of the baseline model (without biomarker) and each biomarker-enhanced model. From top to bottom: change in the predictive performance using likelihood ratio χ^2 (LR), predictive variance (PV), and area under the receiver-operating characteristic curve (AUC). All metrics are increasing with improvement in model fit and predictive ability. Logistic regression was used for model prediction. SBRT = stereotactic body radiation therapy; CBC = complete blood count; GEN = genetic; IPC = inflammatory plasma cytokine; BL=baseline model; GEN=genomic biomarkers (green); IPC=inflammatory plasma cytokine (blue); CBC=complete blood count (red). T2= before fraction 4 of SBRT. Boxplot comparisons were performed using Wilcoxon rank-sum test at significance level 0.05. Significance code = *: < 0.05; **: < 0.01; ***: < 0.001; ****: < 0.0001.*

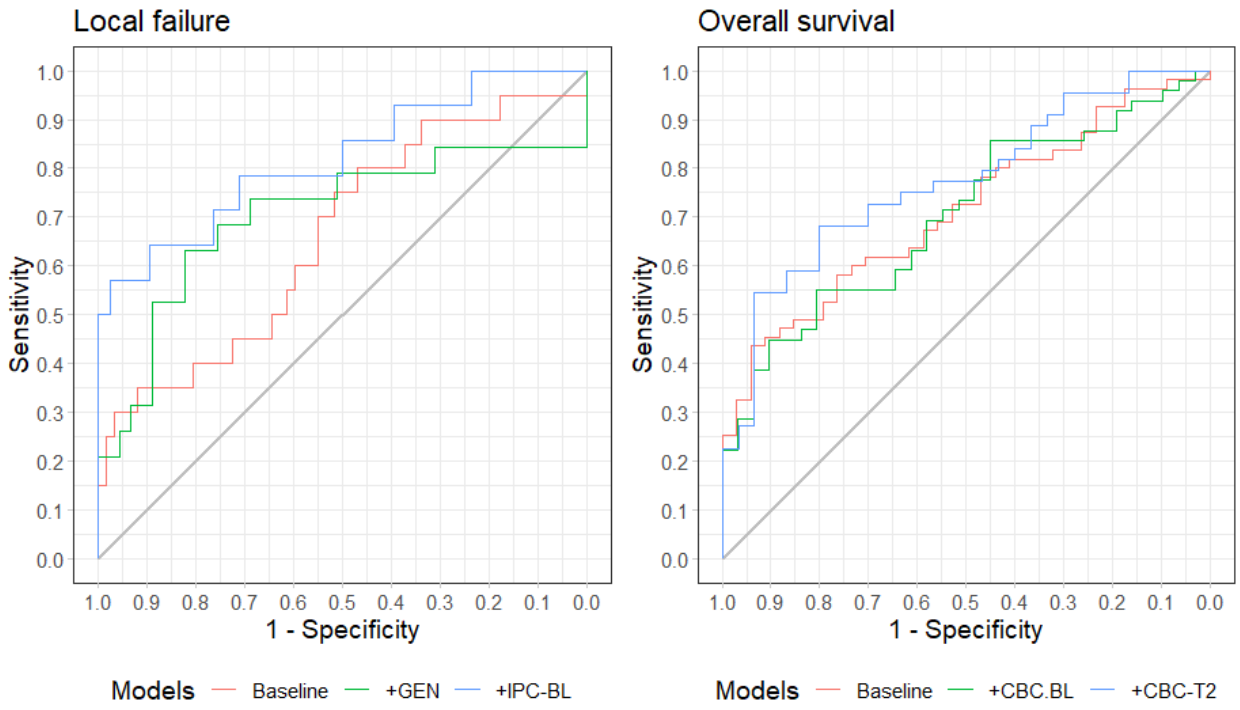

Figure 4. Receiver-operating characteristic (ROC) curves for the predictive models developed for local failure (left) and overall survival (right). The plot shows the incremental added value gained in terms of area under the curve (AUC) by adding each class of biomarkers to the baseline predictors, as reflected in the vertical distance between ROC plots. GEN = genetic biomarkers; CBC = complete blood count; IPC = inflammatory plasma cytokine; T2 = fraction 4 of SBRT; BL = baseline. SBRT = stereotactic body radiation therapy.

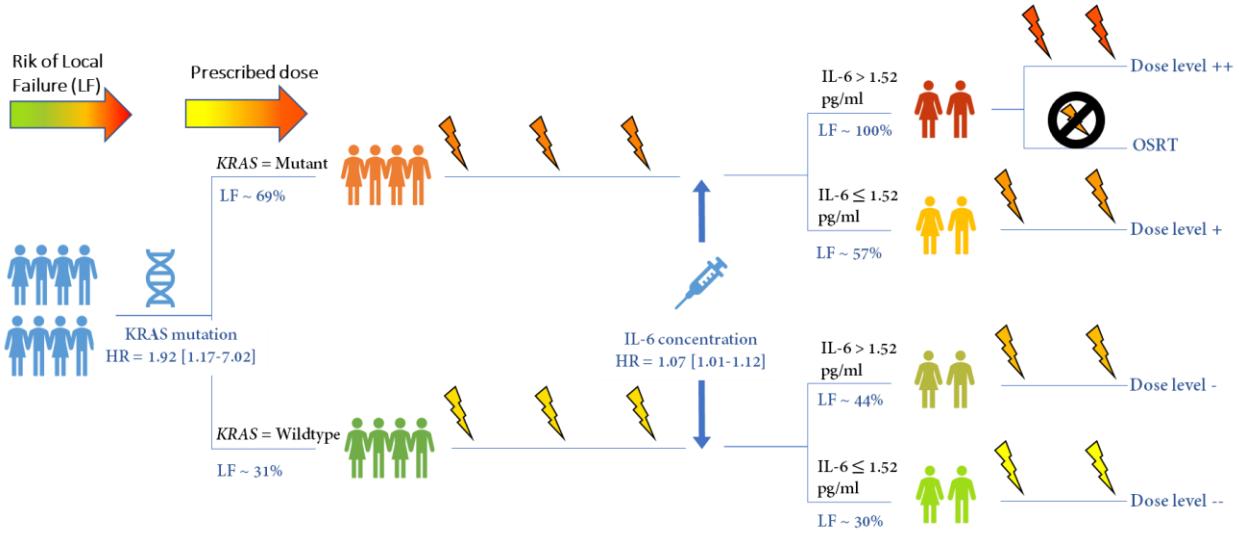

Figure 5. Schematics of dynamic patient stratification using baseline genotype information (i.e., KRAS mutation status) and mid-treatment interleukin (IL) 6 concentration in blood (plasma). The actual local failure (LF) rate is written for each patient group in blue,and is based on a small subset of patients who had both baseline genotype and mid-treatment plasma IL-6 data available (n=36). OSRT, optimal stopping of radiation therapy: for patients who might not benefit from treatment continuation, the treatment might be stopped to spare normal tissue or to switch to an alternative treatment modality (see [1] for details).

Table 1. Patient, treatment, and tumor characteristics. CRC, colorectal cancer; RT, radiotherapy; IQR, inter-quartile range; GTV, gross tumor volume; GyE, Gray equivalent.

Variable grouping	All patients (n = 89)	CRC (n = 34)	# of metastases ≥2 (n = 34)
Demographics			
Age, median (range), years	67.6 (34-88)	72.7 (40-88)	66.06 (45-83)
Sex = Female, No. (%)	33 (37.08)	10 (29.41)	11 (32.35)
Histology/primary site, No. (%)			
CRC	34 (38.2)	34 (100%)	10 (29.41)
Pancreatic	13 (14.61)	N/A	6 (56.15)
Esophagogastric	12 (13.48)	N/A	4 (33.33)
Liver	8 (8.99)	N/A	7 (87.5)
Lung	8 (8.99)	N/A	1 (12.5)
Breast, duodenal, other	14 (15.73)	N/A	6 (42.86)
Prior treatment, No. (%)			
Chemo*	75 (84.27)	32 (94.12)	25 (73.53)
Surgery*	43 (48.31)	26 (76.47)	17 (50.0)
RT*	44 (49.43)	30 (93.75)	24 (70.59)
None	4 (4.49)	0	4 (11.76)
Chemo duration, median (IQR), months	5 (2-12)	5.5 (3-11.75)	4 (0.125-11)
Distance from last systemic therapy, median (IQR), months	2 (1-7)	2 (1-9)	2 (1-3)
Longest tumor size, median (range), cm	3.4 (0.8-11.9)	3.6 (1-11.3)	3.6 (0.8-11)
Size of uninvolved liver, median (range), cc	1593 (800-2577)	1642 (800-2401)	1617 (800-2225)
Effective treatment volume (Veff), median (range)	23.6 (0-93.4)	25.2 (0.2-93.4)	31.5 (0.4-60.8)
Total dose to GTV, No. (%), GyE			
30	7 (7.9)	2 (5.9)	3 (8.8)
40	51 (57.3)	22 (64.7)	25 (73.52)
50	31 (34.8)	10 (29.4)	6 (17.64)

* *Including stand-alone and combination therapy*

Table 2. Analysis of the added predictive value of additional blood biomarkers.

Local failure					
	GEN		IPC		
	BL	BL + GEN	BL	BL + IPC.BL	BL + IPC.T2
LR	9.51	17.17	13.33	26.95	26.95
	[1.45-26.76]	[3.61-39.07]	[2.80-34.90]	[8.02-57.17]	[8.02-57.17]
PV	0.03	0.06	0.04	0.08	0.08
	[0.005-0.086]	[0.02-0.11]	[0.009-0.10]	[0.02-0.13]	[0.02-0.13]
AUC	0.73	0.79	0.77	0.84	0.84
	[0.56-0.88]	[0.64-0.92]	[0.61-0.92]	[0.70-0.96]	[0.70-0.96]
Overall survival					
	GEN		CBC		
	BL	BL + GEN	BL	BL + CBC.BL	BL + CBC.T2
LR	8.86	8.86	10.62	13.09	20.37
	[1.49-20.38]	[1.49-20.38]	[2.27-21.96]	[4.01-27.09]	[8.04-37.88]
PV	0.04	0.04	0.04	0.05	0.07
	[0.008-0.08]	[0.008-0.08]	[0.01-0.08]	[0.02-0.09]	[0.03-0.11]
AUC	0.72	0.72	0.72	0.75	0.80
	[0.58-0.84]	[0.58-0.84]	[0.61-0.83]	[0.63-0.85]	[0.69-0.89]