Plasma and Urine Free Glycosaminoglycans as Monitoring and Predictive Biomarkers in Metastatic Renal Cell Carcinoma: A Prospective Cohort

BIOMARKERS

original reports abstract

Plasma and Urine Free Glycosaminoglycans as Monitoring and Predictive Biomarkers in Metastatic Renal Cell Carcinoma: A Prospective Cohort Study

Francesco Gatto, PhD[1,2]; Sinisa Bratulic, PhD[1]; Eric Jonasch, MD, PhD[3]; Angelo Limeta, MSc[1]; Francesca Maccari, PhD[4]; Fabio Galeotti, PhD[4]; Nicola Volpi, PhD[4]; Sven Lundstam, MD, PhD[5,6]; Jens Nielsen, PhD[1,7]; and Ulrika Stierner, MD, PhD[6]

PURPOSE No liquid biomarkers are approved in metastatic renal cell carcinoma (mRCC) despite the need to predict and monitor response noninvasively to tailor treatment choices. Urine and plasma free glycosaminoglycan profiles (GAGomes) are promising metabolic biomarkers in mRCC. The objective of this study was to explore if GAGomes could predict and monitor response in mRCC.

PATIENTS AND METHODS We enrolled a single-center prospective cohort of patients with mRCC elected for first-line therapy (ClinicalTrials.gov identifier: NCT02732665) plus three retrospective cohorts (ClinicalTrials.gov identifiers: NCT00715442 and NCT00126594) for external validation. Response was dichotomized as progressive disease (PD) versus non-PD every 8-12 weeks. GAGomes were measured at treatment start, after 6-8 weeks, and every third month in a blinded laboratory. We correlated GAGomes with response and developed scores to classify PD versus non-PD, which were used to predict response at treatment start or after 6-8 weeks.

RESULTS Fifty patients with mRCC were prospectively included, and all received tyrosine kinase inhibitors (TKIs). PD correlated with alterations in 40% of GAGome features. We developed plasma, urine, and combined glycosaminoglycan progression scores that monitored PD at each response evaluation visit with the area under the receiving operating characteristic curve (AUC) of 0.93, 0.97, and 0.98, respectively. For internal validation, the scores predicted PD at treatment start with the AUC of 0.66, 0.68, and 0.74 and after 6-8 weeks with the AUC of 0.76, 0.66, and 0.75. For external validation, 70 patients with mRCC were retrospectively included and all received TKI-containing regimens. The plasma score predicted PD at treatment start with the AUC of 0.90 and at 6-8 weeks with the AUC of 0.89. The pooled sensitivity and specificity were 58% and 79% at treatment start. Limitations include the exploratory study design.

CONCLUSION GAGomes changed in association with mRCC response to TKIs and may provide biologic insights into mRCC mechanisms of response.

JCO Precis Oncol 7:e2200361. © 2023 by American Society of Clinical Oncology

INTRODUCTION

There are currently no approved biomarkers for renal cell carcinoma (RCC).[1,2] At the same time, the landscape of systemic therapies available to treat metastatic RCC (mRCC) has dramatically expanded and is still rapidly evolving.[3] Selection of therapy is increasingly relying on prognostic risk models such as the International Metastatic RCC Database Consortium model although these models were not designed to predict response to therapy.[4,5] Because of the lack of liquid or molecular biomarkers, response monitoring relies on medical imaging. However, imaging can only evaluate changes in tumor size and cannot inform on the molecular changes in the tumor, which ultimately may determine progression.[6,7]

There is therefore an urgent need to develop and validate noninvasive diagnostic tests informative of the patient's tumor biology to improve response prediction and monitoring in mRCC. Liquid biopsies belong to an emerging class of cancer diagnostics on the basis of nonsolid tissues (eg, blood), which measure a variety of tumor-related biomarkers, for example, circulating free DNA (cfDNA).[8] Liquid biopsies on the basis of cfDNA have so far shown disappointing yields in mRCC.[9] Recent developments using methylated cfDNA or metabolomics are promising, but the availability of standardized kits remains many years away.[10,11]

We and others observed that genetic alterations specific to RCC correlated with significant reprogramming of cell metabolism.[12-14] Using a systems

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on December 22, 2022 and published at ascopubs.org/journal/po on February 27, 2023: DOI https://doi.org/10.1200/PO.22.00361

CONTEXT

Key Objective

There are currently no approved liquid biomarkers for predicting and monitoring response to therapy in metastatic renal cell carcinoma (mRCC). We examined whether free glycosaminoglycan (GAG) profiles can be used to predict and monitor response in mRCC.

Knowledge Generated

By correlating GAG profiles features in plasma and/or urine with response to therapy, we identified specific features that differentiate responders from nonresponders. The features could then be used to develop GAG progression scores that can quantify a patient's risk for progression. For patients with mRCC receiving tyrosine kinase inhibitors, the GAG progression scores appeared to be useful in predicting progression events in both internal and external cohorts.

Relevance

GAG progression scores could in the future be used by clinicians to identify patients with a high risk of progression and subsequently treat them with another regimen. This would avoid delays in patients receiving effective treatments and minimize unnecessary treatment-related side effects.

biology approach, we specifically identified the deregulation of glycosaminoglycan (GAG) biosynthesis in RCC.[15] GAGs are structurally diverse polysaccharides, and their complex profiles of sulfation and epimerization patterns have been implicated in cancer processes such as tumor growth and invasion.[16,17] Advancements in analytical measurements using mass spectrometry (MS)[18-20] enabled us to appreciate the diversity of GAG profiles (GAGomes).[21] In both a retrospective and prospective series of patients with mRCC and nonmetastatic RCC, plasma and urine GAGomes were significantly altered compared with healthy individuals[15,22] and were found to be prognostic for progression-free and overall survival in mRCC.[23] In addition, we verified that free GAGomes—that is not protein-bound as opposed to total GAGomes as measured in the aforementioned studies—had predictable reference levels in healthy adults[24] and appeared to be useful in the noninvasive surveillance for RCC recurrence.[25] However, it is yet unknown whether free GAGomes can be equally useful to monitor or predict response to therapy in mRCC.

In this study, we explored the correlation of urine and plasma free GAGomes with mRCC response to first-line therapy in a single-center prospective patient cohort followed up longitudinally every three months, concomitantly with the response evaluation visits.

PATIENTS AND METHODS

Study Design

This study is reported in compliance with the STARD guidelines (Data Supplement). The present study obtained ethical permission from the Ethical Committee (Etikprövningsmyndigheten) in Gothenburg, Sweden, in February 2016 (047-16, ClinicalTrials.gov identifier: NCT02732665). No hypotheses on the performance of GAGomes for mRCC response monitoring or prediction could be prespecified at study start given the available prior data,[15] so we designed the study as exploratory.

The study followed a single-center prospective cohort diagnostic design. Key eligibility criteria were diagnosis of mRCC and being elected for first-line drug therapy.

After treatment initiation, patients were followed with radiologic imaging every three months for up to 12 months. The patients provided EDTA-plasma and urine samples at a baseline visit (at treatment start), at the first 6 $\pm$ 2 weeks post-treatment visit, and at the third, sixth, and ninth $\pm$ 1-month response evaluation follow-up visits. Radiologic imaging was performed using chest/abdomen computed tomography. Response was assessed by the investigator (U.S.) as progressive disease (PD) or non-progressive disease (non-PD) on the basis of clinical and radiologic findings. In a subset of patients, an external centralized radiologic review according to RECIST 1.1 was performed to determine concordance with the investigator-assessed response in terms of Cohen's kappa coefficient, wherein $\kappa > 0.6$ was assumed as sufficient concordance.[26]

Free GAGome Measurements

Free GAGome analysis was performed at a single blinded central laboratory (Lablytica Life Sciences AB, Uppsala, Sweden) using the MIRAM Free Glycosaminoglycan Kit (Elypta AB, Stockholm, Sweden), wherein the concentrations (in microgram per milliliter) of 17 chondroitin sulfate (CS), heparan sulfate, and hyaluronic acid disaccharides—that is the free GAGome features—were detected and quantified using a UHPLC-MS/MS system (Acquity I-class Plus Xevo TQ-S micro; Waters Corporation, Milford, MA) following the kit instructions for use.[20] Outlier samples were identified and excluded.

Correlation Analysis of GAG Measurements and Response

Each detectable feature in the plasma or urine free GAGome (> 0.1 μg/mL) was correlated with response as assessed at the same response evaluation follow-up visit in which the corresponding blood or urine sample was collected, that is, at the 3-, 6-, or 9-month follow-up visit. A patient could contribute more than one response. We

regressed response (PD *v* non-PD) as a binary outcome on each detectable GAGome feature, as well as its absolute change from the baseline visit, using a Bayesian mixed effects logistic regression model that included a patient-specific intercept.

Development of GAG Progression Scores

The top two plasma or urine GAGome features were used as inputs to train a Bayesian additive regression trees (BART) model—one for plasma and the other for urine.[27] A third BART model was created, which averaged the outputs of the plasma and urine BART models. The output from each BART model is equivalent to the predicted probability of PD (in percent), and it was named plasma, urine, or combined GAG progression score, respectively.

Internal Validation of GAG Progression Scores

The plasma, urine, and combined GAG progression scores—each defined as a separate index test—were internally validated by predicting response as assessed after 3 months using samples collected at treatment start or after 6 weeks. None of these samples were used for score development. We assessed the discrimination performance in terms of area under the receiving operating characteristic curve (AUC) for the classification of PD versus non-PD. A 95% CI lower bound > 0.50 was the criterion used to assess statistically significance. Next, we determined an optimal test positivity cutoff for each index test and used it to cross-tabulate PD versus non-PD as predicted by the score versus investigator-assessed response. The three resulting confusion matrices (one per score) were used to determine the specificity and sensitivity and the 95% CI.

External Validation of GAG Progression Scores

The plasma GAG progression score—here defined as the index test—was externally validated on three cohorts sourced from two clinical trials (ClinicalTrials.gov identifiers: NCT00126594[28] and NCT00715442[29]). ClinicalTrials.gov identifier: NCT00126594 randomly assigned patients with metastatic clear cell RCC (ccRCC) to first-line sorafenib or sorafenib plus low-dose interferon-α 2b, whereas ClinicalTrials.gov identifier: NCT00715442 treated patients with metastatic ccRCC with sunitinib followed by cytoreductive nephrectomy. The free GAGome was measured at treatment start and after 8 weeks. The plasma GAG progression score was first recalibrated by refitting the plasma BART model parameters on all samples because sodium citrate-plasma, as opposed to EDTA-plasma, was collected from these patients.

We conducted a meta-analysis of the plasma GAG progression score performance by pooling sensitivity and specificity estimates in all four cohorts (retrospective and prospective) using a bivariate linear mixed effect model. All statistical analyses were performed using R (4.0.3).

See the Data Supplement for additional details.

RESULTS

Patient Characteristics

The study design and patient flow are illustrated in Figure 1. Fifty patients with mRCC scheduled for first-line therapy were prospectively enrolled between August 2016 and May 2019 (Data Supplement). The median age at treatment start was 68.5 (range, 46-79) years. The cohort was predominantly composed of males (66%, 33 males *v* 17 females). The most common histologic subtype was ccRCC (n = 46, 92%), followed by three papillary RCC (type 2) and one chromophobe

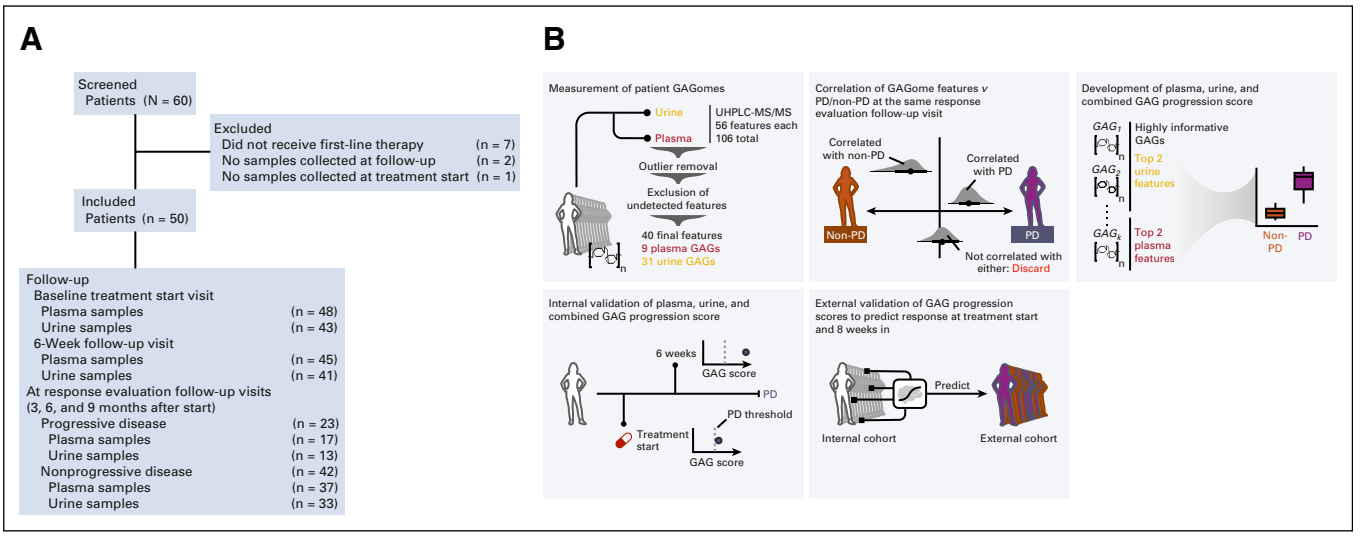

FIG 1. Study design. (A) Patient flow and samples collected at each visit. (B) Workflow used to select free GAGome features correlated with response, to develop GAG progression scores, and to internally and externally validate their use to predict response at two different time points, treatment start and after 6-8 weeks. GAG, glycosaminoglycan; GAGome, glycosaminoglycan profile; non-PD, nonprogressive disease; PD, progressive disease.

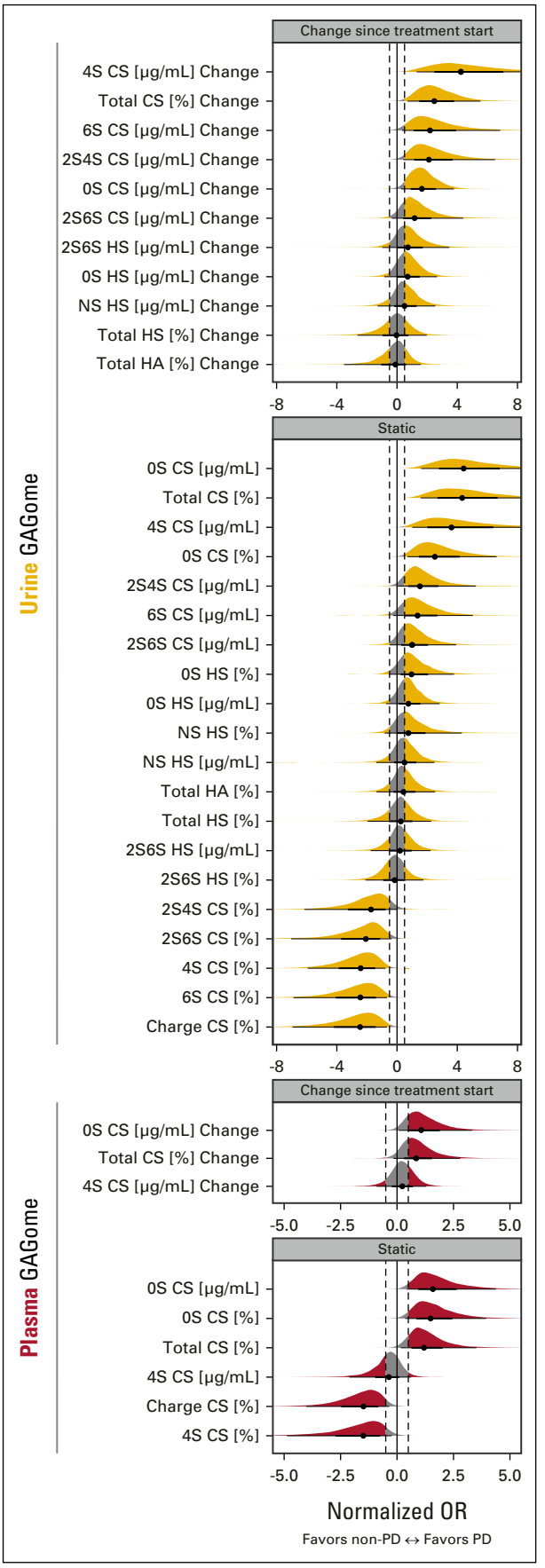

FIG 2. Correlation of detectable plasma and urine free GAGome features (as static measurements or as change from treatment start) in 39 mRCC patients with PD (n = 17) versus non-PD (n = 37) during response evaluation follow-up visits. The posterior probability density of the log OR for PD per unit change of the free GAGome feature (in standard deviation from the mean value) is plotted together with the mean log OR and the 95% credible interval (thick black line). The ROPE is marked by the two vertical dashed lines. A free GAGome feature was deemed compatible with PD or non-PD if its 95% credible interval did not fall inside the ROPE by > 5%. CS, chondroitin sulfate; GAGome, glycosaminoglycan profile; HA, hyaluronic acid; HS, heparan sulfate; mRCC, metastatic renal cell carcinoma; non-PD, nonprogressive disease; OR, odds ratio; PD, progressive disease; ROPE, region of practical equivalence.

RCC. All patients received tyrosine kinase inhibitors (TKIs) as first-line therapy (37 [74%] sunitinib, four [8%] pazopanib, and nine [18%] cabozantinib). Patient prognosis was classified using the International Metastatic RCC Database Consortium model risk model into favorable (n = 8, 16%), intermediate (n = 23, 46%), and poor (n = 16, 32%)—three (6%) patients were not classifiable. Centralized review of radiologic response was performed in a subset of patients in the first 3-month response evaluation follow-up visit (n = 29, 58%). The concordance with the investigator assessment was substantial (86% agreement, Cohen's kappa coefficient = 0.66).

The median follow-up time was 3.6 (range, 1-8) months. Each patient contributed on average 1.3 response evaluation follow-up visits and 65 in total. Throughout these, we recorded 23 (35%) PD and 42 non-PD (65%). In the subset of 29 patients with centralized review, we recorded seven (24%) partial response (PR), 12 (41%) stable disease (SD), and 10 (35%) PD. Plasma and urine samples were obtained in 49 and 43 patients at treatment start and 46 and 41 patients at 6 weeks and in 54 and 46 response evaluation follow-up visits, respectively. No adverse events were recorded during sample collection or response evaluation.

Correlation Between GAG Profiles and Radiologic Response

We measured the free GAGome in 279 samples, either plasma or urine, in a single-blind central laboratory using a standardized UHPLC-MS/MS kit. One (< 1%) plasma sample was identified as an outlier and excluded.

We first explored the correlation between free GAGome features and response using samples obtained at the same response evaluation follow-up visit. In this analysis, we thus excluded treatment start and 6-week samples. In this set of 39 patients, each patient contributed an average of 1.4 response evaluation follow-up visits totaling 54 responses, 17 PD and 37 non-PD. Plasma and urine free GAGomes were determined in 100% and 85% of all visits, respectively.

We found that nine of 56 plasma free GAGome features and 31 of 56 urine free GAGome features were detectable. Three (33%) of nine detectable features in plasma and 13 (42%) of 31 detectable features in urine were compatible with PD versus non-PD (Fig 2 and Data Supplement). In general, PD tended to correlate with free GAGomes featuring a higher concentration of nonsulfated CS (0S CS), a higher level of total CS, and conversely a relatively lower concentration of monosulfated CS, particularly (4S CS). Overall, we observed that treatment response was compatible with alterations in 40% of detectable free GAGome features.

Development of Plasma, Urine, and Combined GAG Progression Scores to Monitor Response in mRCC

We trained two BART models to regress PD versus non-PD on the top two plasma or the top two urine free GAGome features. The models' output, henceforth referred to as plasma or urine GAG progression score, was scaled between 0 and 100 so that a score > 50 indicated a probability of PD > 50% according to the model. We developed the combined GAG progression score by averaging the plasma and urine scores when both samples were available.

For the classification of PD versus non-PD, the AUC was 0.93 (95% CI, 0.87 to 0.99), 0.97 (95% CI, 0.94 to 1), and 0.98 (95% CI, 0.95 to 1) for the plasma, urine, and combined GAG progression score, respectively (Fig 3 and Data Supplement). Using an optimal cutoff to determine PD, the sensitivity and specificity were 94% and 84% in plasma, 92% and 91% in urine, and 85% and 97% when combined (Data Supplement). We did not observe evident changes in the performance of the scores in the subset of 27 patients with ccRCC treated with sunitinib only (Data Supplement) nor in the subset of four non-ccRCC patients (Data Supplement).

Subset Analysis of GAG Progression Scores Versus Centralized Radiologic Response

In a subset of 29 patients with centralized review, we compared the scores computed at the 3-month visit with PR versus SD versus PD according to RECIST 1.1. Of 29, the objective responses of 22 patients with either plasma or urine samples were six PD, 12 SD, and four PR.

We observed a weak yet compatible correlation between plasma, urine, and combined GAG progression scores and the percentual change from the nadir in the sum of diameters of the target lesions (Bayesian $R^2 = 0.12$, 0.16, and

0.13, respectively; Fig 4). Notably, we observed a stepwise increase in all scores from PR to SD to PD.

Internal Validation of GAG Progression Scores to Predict Response at Treatment Start or After 6 Weeks

We opted to validate the GAG progression scores internally by predicting response at two time points: at treatment start, to aid the selection of TKI agents as first-line therapy, and at 6 weeks, when no radiologic evaluation is routinely available.

At treatment start, the test was performed in 50 patients. Seventeen (33%) patients had PD at the following 3-month response evaluation visit, and 33 (66%) had non-PD. Plasma and urine free GAGomes were measured in 49 (98%) and 43 (86%) patients, respectively. The AUC for PD versus non-PD was 0.66 (95% CI, 0.50 to 0.83), 0.68 (95% CI, 0.48 to 0.88), and 0.74 (95% CI, 0.57 to 0.90) using plasma, urine, or combined GAG progression score, respectively (Data Supplement). At an optimal cutoff, the sensitivity and specificity to PD versus non-PD were 47% and 81% in plasma, 54% and 87% in urine, and 62% and 90% when combined (Fig 5 and Data Supplement).

At 6 weeks, the test was performed in 47 patients. Fifteen (32%) patients had PD at the following 3-month response evaluation visit, and 32 (68%) had non-PD. Plasma and urine free GAGomes were measured in 46 (98%) and 41 (87%) patients, respectively. The AUC for PD versus non-PD was 0.76 (95% CI, 0.60 to 0.92), 0.66 (95% CI, 0.47 to 0.86), and 0.75 (95% CI, 0.59 to 0.91) using plasma, urine, or combined GAG progression score, respectively (Data Supplement). At an optimal cutoff, the sensitivity and specificity to PD versus non-PD were 53% and 81% in plasma, 53% and 88% in urine, and 67% and 80% when combined (Fig 5 and Data Supplement).

External Validation of GAG Progression Scores to Predict Response at Treatment Start or After 6 Weeks

We sought to validate the plasma GAG progression score in three external cohorts by predicting response at treatment start and at 8 weeks in patients included in treatment arms of the ClinicalTrials.gov identifiers: NCT00126594 and NCT00715442 trials (Data Supplement). In total, we included 70 patients with clear cell mRCC treated with first-line sorafenib (n = 24), sorafenib plus interferon-α 2b (n = 18), or sunitinib plus cytoreductive nephrectomy (n = 28). Plasma free GAGomes were measured in 70 (100%) patients at treatment start and 69 (99%) at 8 weeks.

At treatment start, the test was performed in 70 patients. Thirteen (19%) patients had PD at the following 3-month visit, and 57 (81%) had non-PD. The AUC for PD versus non-PD was 0.90 (95% CI, 0.82 to 0.98; Data Supplement). At an optimal cutoff, the sensitivity and specificity to PD versus non-PD were 92% and 81% (Fig 6 and Data Supplement).

At 8 weeks, the test was performed in 69 patients. Twelve (17%) patients had PD at the following 3-month visit, and 57 (83%) had non-PD. The AUC for PD versus non-PD was 0.89 (95% CI, 0.81 to 0.97). At an optimal cutoff, the sensitivity and specificity to PD versus non-PD were 75% and 82% (Fig 6 and Data Supplement). The diagnostic performance per cohort is reported in the Data Supplement.

A meta-analysis of the plasma GAG progression score across all four cohorts in this study estimated 58% sensitivity (95% CI, 39 to 72) and 79% specificity (95% CI, 70 to 86) to PD at treatment start (n_{tot} = 119) and 59% sensitivity (95% CI, 40 to 76) and 79% specificity (95% CI, 69 to 86) at 6-8 weeks (n_{tot} = 115).

Finally, we observed that the correlations between the plasma free GAGome features and response reported in Figure 2 appeared to be recapitulated across the three external cohorts at both treatment start (Data Supplement) and after 6-8 weeks (Data Supplement).

DISCUSSION

In this study, we explored the use of plasma versus urine free GAGomes as novel liquid biomarkers to monitor and

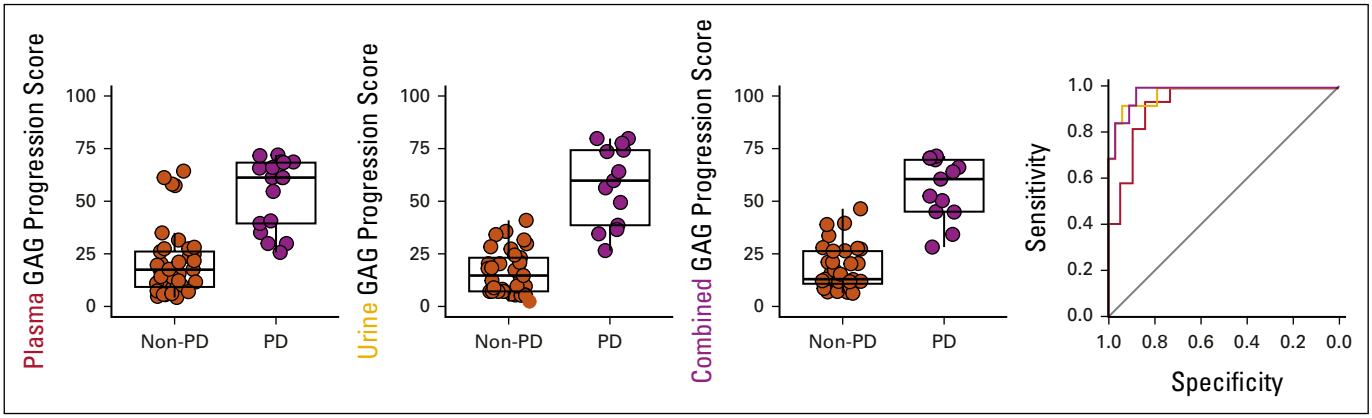

FIG 3. Development of plasma, urine, and combined GAG progression score in mRCC at the response evaluation follow-up visits and their corresponding receiver operating characteristic curves (n = 39 patients, 17 PD *v* 37 non-PD for plasma; 35 patients, 13 PD *v* 33 non-PD for urine and combined). GAG, glycosaminoglycan; mRCC, metastatic renal cell carcinoma; non-PD, nonprogressive disease; PD, progressive disease.

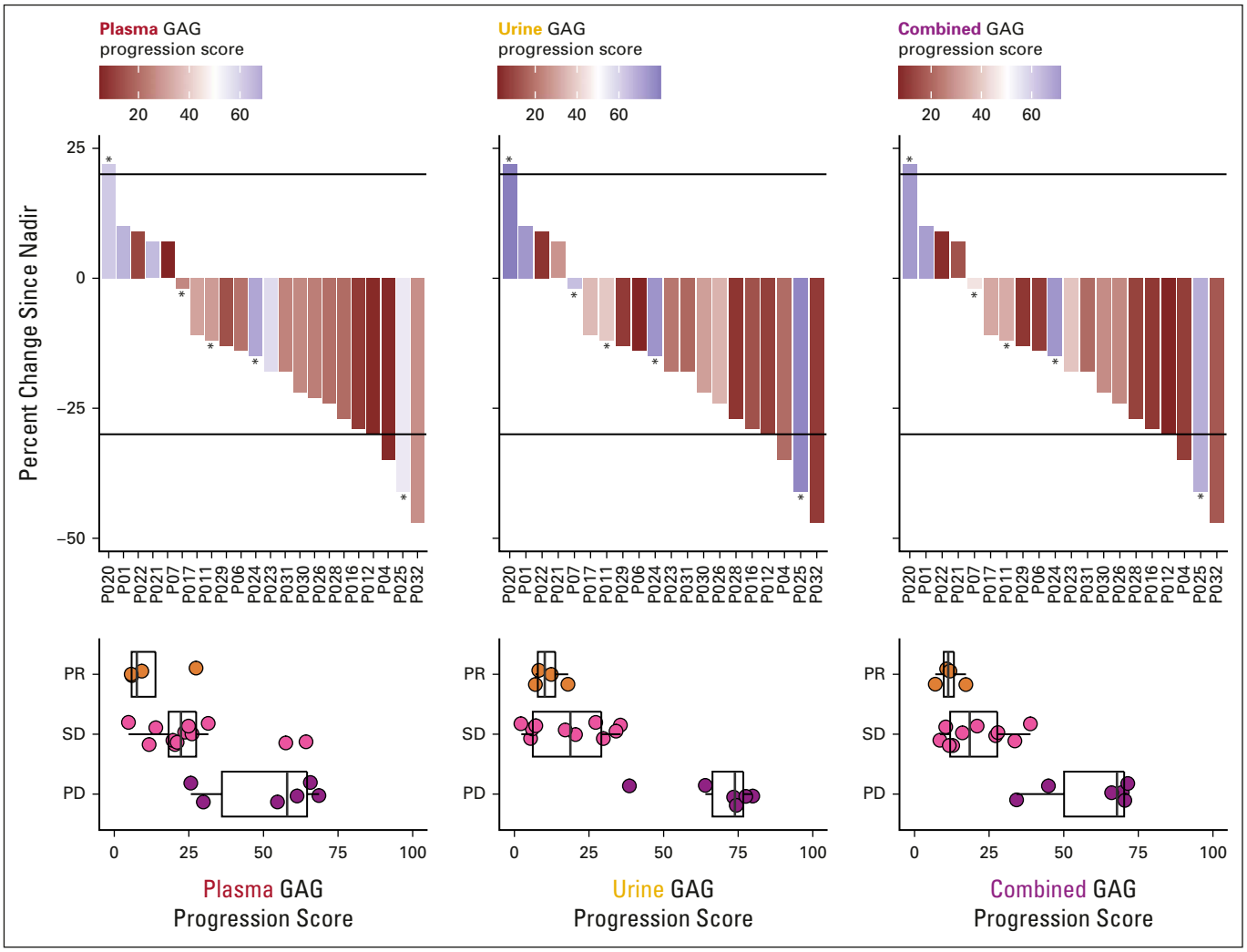

FIG 4. (Top) Waterfall plot for the percentage change from the nadir in the sum of diameters of target lesions in the first 3-month response evaluation color-coded by plasma, urine, and combined GAG progression score (n = 22 patients for plasma and 20 for urine and combined). Unequivocal new lesions are marked with a star. (Bottom) Plasma, urine, and combined GAG progression scores by radiologic response according to RECIST 1.1 at the first 3-month response evaluation visit (n_{PD} = 6, n_{SD} = 12, and n_{PR} = 4). GAG, glycosaminoglycan; PD, progressive disease; PR, partial response; SD, stable disease.

predict response in mRCC. Collectively, our results indicate that free GAGomes do provide information at the molecular level—presumably reflective of RCC metabolism—that correlated with treatment response on the same visit as the radiologic evaluations, particularly when measured in the plasma or when combining plasma and urine measurements. We observed that these correlations were also valid at earlier time points, such as treatment start or after 6-8 weeks. Overall, the information captured in GAG progression scores appears to be useful to predict and monitor response to therapy in mRCC.

From a biological standpoint, we observed that PD implicated an increase in nonsulfated CS in both plasma and urine and accordingly a relative decrease in monosulfated CS concentration, particularly 4S CS. These changes were consistent with our previous study on RCC recurrence versus no evidence of disease after curative-intent surgery.[25] In urine, but not plasma, these changes were also consistent with our previous study in RCC versus healthy controls[15] although our previous study measured total and not free GAGomes. This is likely indicative of negligible levels of protein-bound GAGomes in urine (but not plasma), making historical results comparable. Overall, it appears that RCC progression induces an increase in circulating nonsulfated CS over monosulfated CS. This CS could originate from the tumor itself through de novo biosynthesis,[30] possibly modifying the biophysical properties of the extracellular matrix[31,32] or inducing epithelial–mesenchymal transition[33] or causing inflammation[34] promoting growth and invasion. Another hypothesis is that free GAGome changes with PD are induced by successful evasion of antiangiogenic therapy,

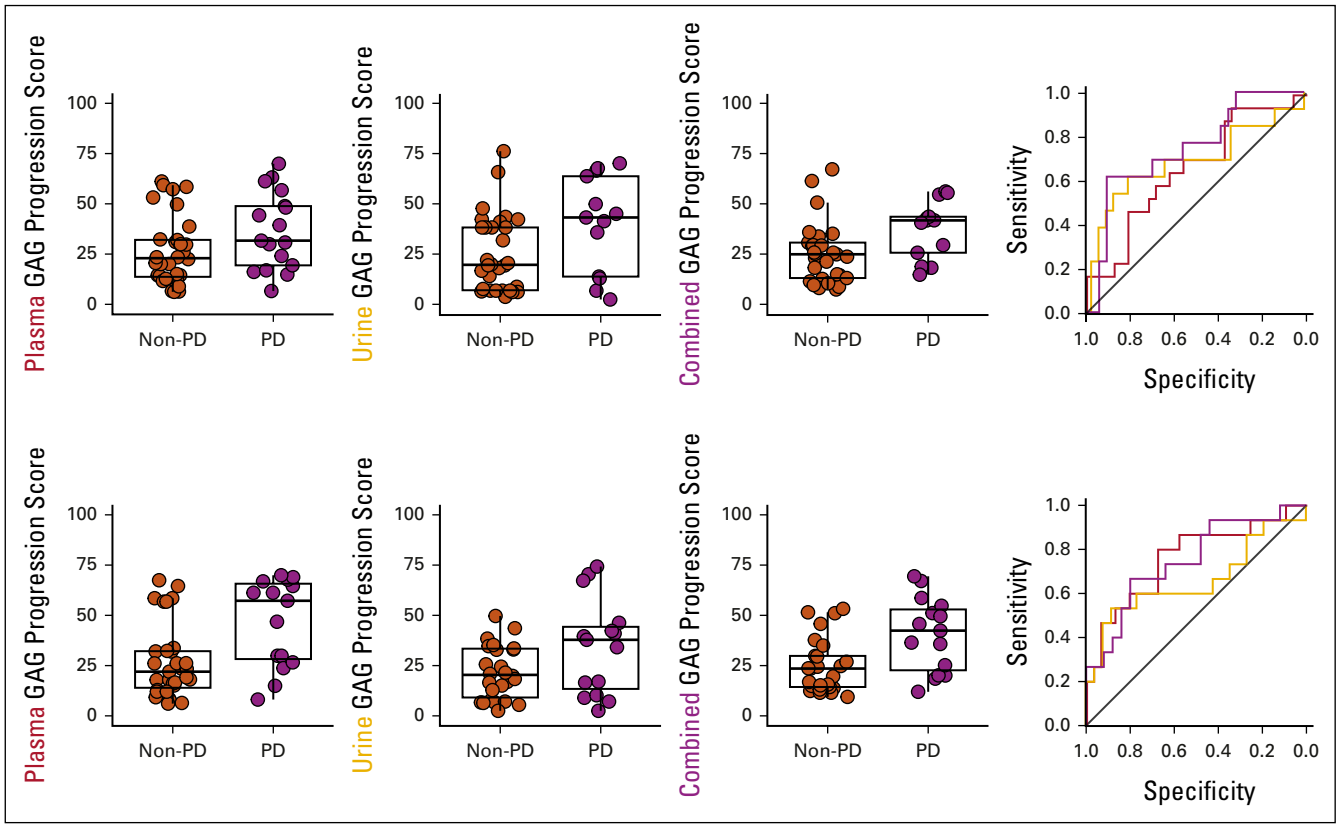

FIG 5. Internal validation of plasma, urine, and combined GAG progression scores to predict PD versus non-PD at treatment start (top, n = 50, 17 PD *v* 33 non-PD, n_{plasma} = 49, n_{urine} = 43, $n_{combined}$ = 42) and at 6 weeks (bottom, n = 47, 15 PD *v* 32 non-PD, n_{plasma} = 46, n_{urine} = 41, $n_{combined}$ = 40) and their corresponding receiver operating characteristic curves. GAG, glycosaminoglycan; non-PD, nonprogressive disease; PD, progressive disease.

which was previously associated with increased CS in cell cultures.[35]

Many liquid biomarkers have been explored in connection with treatment response in mRCC, including metabolomics,[11] vascular endothelial-cadherin,[36] the neutrophil-to-lymphocyte ratio,[37] and circulating tumor DNA.[38,39] However, most of these studies were performed in retrospective cohorts. Therefore, none of them was designed prospectively to report the sensitivity and specificity to PD as the primary end point. We believe that the main strength of this study was its prospective single-center cohort design. This design allowed for the recruitment of a homogeneous and moderately sized population with high compliance to sample collection while limiting biases that could confound the correlations between GAGomes and response, which indeed appeared to be recapitulated in external cohorts. Another advantage was the

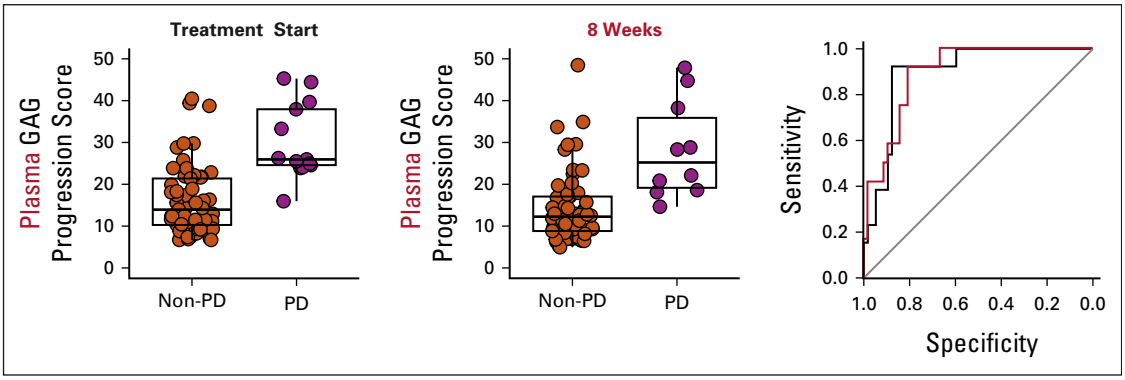

FIG 6. External validation of the plasma GAG progression score to predict PD versus non-PD at treatment start (n = 70, 13 PD *v* 57 non-PD) and at 8 weeks (n = 69, 12 PD *v* 57 non-PD) across three cohorts, and their corresponding receiver operating characteristic curves. GAG, glycosaminoglycan; non-PD, nonprogressive disease; PD, progressive disease.

availability of standardized UHPLC-MS/MS kits, with known analytical performance characteristics and reference ranges in healthy individuals.[20,24] Thus, we predict that the translational gap for clinical implementation could be shorter than other exploratory biomarkers.

The study has limitations. First, we did not report evidence of performance in patients treated with immune checkpoint inhibitors, which became the first-line recommendation for mRCC while this study was being executed. This limits the generalizability of the results to patients treated with TKIs. Second, the study had no prespecified hypotheses having been conceived as exploratory, and the statistical analysis did not prespecify certain test parameters, such as the cutoff or the model recalibration when sodium citrate plasma was used to compute the score. These issues potentially introduce bias in the estimation of the sensitivity and specificity. For example, the score recalibration might have overestimated performance in the external validation cohorts. In addition, the relatively small event size (33 PDs across all cohorts) affected the precision of these estimates. Finally, the study was a single-center experience and the validation cohorts enrolled patients treated with different therapies. Consequently, the performance of GAG progression scores should not be generalized for routine clinical use even in patients with TKI-treated mRCC until an external validation study on an independent patient population is performed.

The diagnostic performance of the plasma GAG progression score—ultimately tested here in 119 patients—did not appear to differ between four different TKI-containing regimens, nor in patients with non–clear cell histology (although these subsets were not adequately large for precise estimations). Considering this, we speculate that the plasma score once validated in an independent population could be useful to predict and monitor PD in a TKI-agnostic clear cell mRCC population (58% sensitivity and 79% specificity) with promising negative predictive value—ranging 74%-98%—but limited positive predictive value, ranging 47%-73%. This performance is comparable with that of US Food and Drug Administration–approved biomarkers, such as serum cancer antigen 19-9 to monitor progression in pancreatic cancer (64% sensitivity and 60% specificity).[40]

In conclusion, plasma and urine free GAGomes were informative of treatment response in mRCC and may provide new biological insights into the mRCC mechanism of response. The clinical utility of GAG progression scores to predict or monitor response and their therapy agnosticism remain to be ascertained.

AFFILIATIONS

[1]Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[2]Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden
[3]Division of Cancer Medicine, Department of Genitourinary Medical Oncology, MD Anderson Cancer Center of the University of Texas, Houston, TX
[4]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[5]Department of Urology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[6]Department of Oncology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[7]BioInnovation Institute, Copenhagen, Denmark

CORRESPONDING AUTHOR

Francesco Gatto, PhD, Department of Oncology-Pathology, Karolinska Institute, 171 77 Stockholm, Sweden; e-mail: francesco.gatto@ki.se.

EQUAL CONTRIBUTION

J.N. and U.S. contributed equally to this work.

PRIOR PRESENTATION

Presented at 2021 ASCO Annual Meeting, virtual, June 4-8, 2021.

SUPPORT

Supported by the Knut and Alice Wallenberg Foundation (Grant No. 2017.0328 and 2018.0266), Cancerfonden (Grant No. 17.0625), and Ingabritt och Arne Lundbergs Forskningsstiftelse (Grant No. 2016.0011 and 2020.0023) to Chalmers University of Technology (J.N.) and by VINNOVA (Grant No. 2016.00763) and Västra Götaland Region to Chalmers University of Technology (F.G.).

AUTHOR CONTRIBUTIONS

Conception and design: Francesco Gatto, Sven Lundstam, Jens Nielsen, Ulrika Stierner
Financial support: Francesco Gatto, Jens Nielsen
Provision of study materials or patients: Eric Jonasch, Sven Lundstam, Ulrika Stierner
Collection and assembly of data: Francesca Maccari, Fabio Galeotti, Nicola Volpi, Sven Lundstam, Ulrika Stierner
Data analysis and interpretation: Francesco Gatto, Sinisa Bratulic, Eric Jonasch, Angelo Limeta, Sven Lundstam, Jens Nielsen, Ulrika Stierner
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Francesco Gatto
Employment: Elypta
Leadership: Elypta
Stock and Other Ownership Interests: Elypta
Travel, Accommodations, Expenses: Elypta

Sinisa Bratulic
Employment: Elypta

Eric Jonasch
Consulting or Advisory Role: Pfizer, Novartis, Exelixis, Eisai, Aravive, Merck, NiKang Therapeutics, Calithera Biosciences, Takeda, Aveo
Research Funding: Novartis, Merck, NiKang Therapeutics, Arrowhead Pharmaceuticals (Inst)
Travel, Accommodations, Expenses: Pfizer

Sven Lundstam
Consulting or Advisory Role: Ipsen

Jens Nielsen
Stock and Other Ownership Interests: Elypta
Patents, Royalties, Other Intellectual Property: I am an inventor of several patents related to cancer biomarkers. All are licensed to Elypta AB

Ulrika Stierner
Consulting or Advisory Role: Ipsen

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

The authors would like to thank Peter Ueda (Karolinska Institute) for a critical review of the manuscript and Ulla Delle (Sahlgrenska Academy) for assisting in the clinical study execution.

REFERENCES

1. Jonasch E, Futreal PA, Davis IJ, et al: State of the science: An update on renal cell carcinoma. Mol Cancer Res 10:859-880, 2012
2. Moch H, Srigley J, Delahunt B, et al: Biomarkers in renal cancer. Virchows Arch 464:359-365, 2014
3. Kotecha RR, Motzer RJ, Voss MH: Towards individualized therapy for metastatic renal cell carcinoma. Nat Rev Clin Oncol 16:621-633, 2019
4. Escudier B, Porta C, Schmidinger M, et al: Renal cell carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 30:706-720, 2019
5. Motzer RJ, Jonasch E, Agarwal N, et al: Kidney Cancer, Version 2.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 15(6): 804-834, 2017
6. de Velasco G, Krajewski KM, Albiges L, et al: Radiologic heterogeneity in responses to anti–PD-1/PD-L1 therapy in metastatic renal cell carcinoma. Cancer Immunol Res 4:12-17, 2016
7. Pignon JC, Jegede O, Shukla SA, et al: irRECIST for the evaluation of candidate biomarkers of response to nivolumab in metastatic clear cell renal cell carcinoma: Analysis of a phase II prospective clinical trial. Clin Cancer Res 25:2174-2184, 2019
8. Bratulic S, Gatto F, Nielsen J: The translational status of cancer liquid biopsies. Regen Eng Transl Med 7:312-352, 2019
9. Lasseter K, Nassar AH, Hamieh L, et al: Plasma cell-free DNA variant analysis compared with methylated DNA analysis in renal cell carcinoma. Genet Med 22:1366-1373, 2020
10. Nuzzo PV, Berchuck JE, Korthauer K, et al: Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. Nat Med 26:1041-1043, 2020
11. Li H, Bullock K, Gurjao C, et al: Metabolomic adaptations and correlates of survival to immune checkpoint blockade. Nat Commun 10:4346, 2019
12. Gatto F, Nookaew I, Nielsen J: Chromosome 3p loss of heterozygosity is associated with a unique metabolic network in clear cell renal carcinoma. Proc Natl Acad Sci USA 111:E866-E875, 2014
13. Hakimi AA, Reznik E, Lee CH, et al: An integrated metabolic atlas of clear cell renal cell carcinoma. Cancer Cell 29:104-116, 2016
14. The Cancer Genome Atlas Research Network: Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 499:43-49, 2013
15. Gatto F, Volpi N, Nilsson H, et al: Glycosaminoglycan profiling in patients' plasma and urine predicts the occurrence of metastatic clear cell renal cell carcinoma. Cell Rep 15:1822-1836, 2016
16. Afratis N, Gialeli C, Nikitovic D, et al: Glycosaminoglycans: Key players in cancer cell biology and treatment. FEBS J 279:1177-1197, 2012
17. Wei J, Hu M, Huang K, et al: Roles of proteoglycans and glycosaminoglycans in cancer development and progression. Int J Mol Sci 21:5983, 2020
18. Volpi N, Linhardt RJ: High-performance liquid chromatography-mass spectrometry for mapping and sequencing glycosaminoglycan-derived oligosaccharides. Nat Protoc 5:993-1004, 2010
19. Tomatsu S, Shimada T, Mason RW, et al: Assay for glycosaminoglycans by tandem mass spectrometry and its applications. J Anal Bioanal Tech 2014:006, 2014 (suppl 2)
20. Tamburro D, Bratulic S, Abou Shameh S, et al: Analytical performance of a standardized kit for mass spectrometry-based measurements of human glycosaminoglycans. J Chromatogr B Analyt Technol Biomed Life Sci 1177:122761, 2021
21. Chen YH, Narimatsu Y, Clausen TM, et al: The GAGOme: A cell-based library of displayed glycosaminoglycans. Nat Methods 15:881-888, 2018
22. Gatto F, Blum KA, Hosseini SS, et al: Plasma glycosaminoglycans as diagnostic and prognostic biomarkers in surgically treated renal cell carcinoma. Eur Urol Oncol 1:364-377, 2018
23. Gatto F, Maruzzo M, Magro C, et al: Prognostic value of plasma and urine glycosaminoglycan scores in clear cell renal cell carcinoma. Front Oncol 6:253, 2016
24. Bratulic S, Limeta A, Maccari F, et al: Analysis of normal levels of free glycosaminoglycans in urine and plasma in adults. J Biol Chem 298:101575, 2022
25. Gatto F, Dabestani S, Bratulic S, et al: Plasma and urine free glycosaminoglycans as monitoring biomarkers in nonmetastatic renal cell carcinoma—A prospective cohort study. Eur Urol Open Sci 42:30-39, 2022
26. Interrater Reliability: The Kappa Statistic. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3900052/
27. Chipman HA, George EI, McCulloch RE: BART: Bayesian additive regression trees. Ann Appl Stat 4:266-298, 2010
28. Jonasch E, Corn P, Pagliaro LC, et al: Upfront, randomized, phase 2 trial of sorafenib versus sorafenib and low-dose interferon alfa in patients with advanced renal cell carcinoma: Clinical and biomarker analysis. Cancer 116:57-65, 2010
29. Powles T, Blank C, Chowdhury S, et al: The outcome of patients treated with sunitinib prior to planned nephrectomy in metastatic clear cell renal cancer. Eur Urol 60:448-454, 2011
30. Ucakturk E, Akman O, Sun X, et al: Changes in composition and sulfation patterns of glycoaminoglycans in renal cell carcinoma. Glycoconj J 33:103-112, 2016
31. Basu A, Patel NG, Nicholson ED, et al: Spatiotemporal diversity and regulation of glycosaminoglycans in cell homeostasis and human disease. Am J Physiol Cell Physiol 322:C849-C864, 2022
32. Moran H, Cancel LM, Huang P, et al: Glycocalyx mechanotransduction mechanisms are involved in renal cancer metastasis. Matrix Biol Plus 13:100100, 2022
33. Pan H, Xue W, Zhao W, Schachner M: Expression and function of chondroitin 4-sulfate and chondroitin 6-sulfate in human glioma. FASEB J 34:2853-2868, 2020

34. Macke EL, Henningsen E, Jessen E, et al: Loss of chondroitin sulfate modification causes inflammation and neurodegeneration in skt mice. Genetics 214:121-134, 2020
35. Jan SL, Hayashi M, Kasza Z, et al: Functional overlap between chondroitin and heparan sulfate proteoglycans during VEGF-induced sprouting angiogenesis. Arterioscler Thromb Vasc Biol 32:1255-1263, 2012
36. Polena H, Creuzet J, Dufies M, et al: The tyrosine-kinase inhibitor sunitinib targets vascular endothelial (VE)-cadherin: A marker of response to antitumoural treatment in metastatic renal cell carcinoma. Br J Cancer 118:1179-1188, 2018
37. Templeton AJ, Knox JJ, Lin X, et al: Change in neutrophil-to-lymphocyte ratio in response to targeted therapy for metastatic renal cell carcinoma as a prognosticator and biomarker of efficacy. Eur Urol 70:358-364, 2016
38. Pal SK, Sonpavde G, Agarwal N, et al: Evolution of circulating tumor DNA profile from first-line to subsequent therapy in metastatic renal cell carcinoma. Eur Urol 72:557-564, 2017
39. Maia MC, Bergerot PG, Dizman N, et al: Association of circulating tumor DNA (ctDNA) detection in metastatic renal cell carcinoma (mRCC) with tumor burden. Kidney Cancer 1:65-70, 2017
40. U. S. Food and Drug Administration, Center for Devices and Radiological Health: 510(K) substantial equivalence determination decision summary no. K191973, https://www.accessdata.fda.gov/cdrh_docs/reviews/K191973.pdf