Journal of Gastrointestinal Cancer (2019) 50:801–807
https://doi.org/10.1007/s12029-018-0153-9

ORIGINAL RESEARCH

Tumor Platinum Concentrations and Pathological Responses Following Cisplatin-Containing Chemotherapy in Gastric Cancer Patients

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Published online: 16 August 2018

Abstract

Purpose There is a wide range in tumor response following preoperative chemotherapy in locally advanced gastric or gastro-esophageal junction cancers. We investigated the relationship between tumor platinum levels and pathological responses in these patients.

Methods Tumor and adjacent normal tissues were retrieved. Pathological responses were assessed per standard criteria. Tissue platinum concentrations were determined with high-performance liquid chromatography mass spectrometry. Platinum distribution in tissue components was evaluated with imaging mass cytometry. Collagen content was evaluated using trichrome staining.

Results Surgical specimens from 10 patients were available. Surgery was performed at a median time of 49 days (range: 28–72) after the last cycle of chemotherapy. The mean platinum level in tumor tissue in patients with any response was significantly higher than in those with no response (893 ± 460 vs. 38.8 ± 8.8 pg, $P = 0.007$), so was the collagen content (37.4 ± 6.8 vs. 11.5 ± 8.6%, $P < 0.05$). Platinum preferentially bound to collagen.

Conclusions Platinum was detectable in surgical specimens up to 72 days after preoperative chemotherapy. Higher tumor platinum concentration correlated with improved pathological response. Collagen binding potentially explained the high interpatient variability in tumor platinum concentrations.

Keywords Stomach neoplasms · Neoadjuvant therapy · Platinum · Treatment outcome · Image cytometry

Introduction

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer death in the world, with an estimated incidence of 12.1 per 100,000 [1]. In the past decade, perioperative chemotherapy has become a standard treatment for patients with resectable gastric or gastroesophageal junction (GEJ) cancers [2, 3]. In a randomized phase III study, perioperative chemotherapy with 3 cycles of epirubicin, cisplatin, and 5-fluorouracil (ECF) preoperatively and 3 cycles of ECF postoperatively was associated with significantly improved overall survival (hazard ratio [HR]: 0.75; 95% CI: 0.60 to 0.93, $P = 0.009$) [2]). However, only 65.6% patients were able to start the planned chemotherapy postoperatively, and 41.6% actually completed all three scheduled cycles. Similar findings were reported from two more recent randomized phase III studies. Cunningham et al. reported that 546 of 1063 patients (51.4%) started postoperative chemotherapy in a phase III study comparing ECX (epirubicin, cisplatin, and capecitabine [Xeloda®]) vs ECX plus bevacizumab [4]. Al-Batran et al. compared ECF/ECX versus docetaxel, oxaliplatin, and 5-fluorouracil or capecitabine (FLOT) in the same patient population [3]. Fifty-two percent of patients were able to start postoperative chemotherapy and 37% completed all 3 cycles of postoperative ECF/ECX, while 60% were able to start and 46% completed all 4 cycles of postoperative FLOT. Given difficulties with delivering postoperative

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chemotherapy, it is important to identify predictive and prognostic markers to guide the decision-making process postoperatively.

Other than TNM stage, there is no validated predictive marker for survival in gastric or GEJ cancer patients. The extent of pathological tumor response following preoperative chemotherapy has been investigated as a predictor for survival [5, 6]. Smyth et al. recently reported the relationship between pathological tumor response and survival following preoperative chemotherapy with ECF/ECX. Although the pathological tumor response was associated with improved survival on univariate analysis, only the lymph node status was predictive of survival in multivariate analysis [5]. Blackham et al. showed similar findings that the pathological response did not predict survival [6].

Cisplatin is widely used as a chemotherapeutic agent. Unlike other drugs, cisplatin and other platinum-containing agents can persist in the body for an extended period after therapy [7]. This unique property makes these agents ideal for investigating drug distribution in tumor tissues following preoperative chemotherapy and for evaluating tissue platinum concentrations as potential biomarkers. Objectives of this study were to evaluate platinum distribution in tumor tissues following preoperative chemotherapy in patients with gastric or GEJ cancer and to investigate the relationship between the extent of pathological tumor response and tissue platinum concentration.

Materials and Methods

Patients and Tissue Specimens

A retrospective chart review was performed. The most recent 10 patients with gastric or GEJ adenocarcinoma who were treated with perioperative chemotherapy with ECF/ECX prior to 2017 at Princess Margaret Cancer Centre, Toronto, Canada, were identified. All patients underwent computer tomography (CT) assessment of disease prior to and after completion of preoperative chemotherapy. Tumor response was determined using RECIST 1.1 criteria. Tumor and adjacent normal tissue sections in 5 μm thickness were cut from formalin-fixed, paraffin-embedded (FFPE) surgical specimens. Demographic- and treatment-related information was collected, including gender, age, tumor site, number of cycles of preoperative chemotherapy, total cisplatin doses received, radiological response, and interval from last cycle of chemotherapy to surgery. The study was approved by the institutional research ethics review board.

The pathological tumor response was assessed based on the tumor regression grade (TRG) system recommended by College of American Pathologists [8]. This TRG system is based on the volume of residual tumor cells, with grade 0 representing complete response, grade 1 moderate response, grade 2 minimal response, and grade 3 no response.

For platinum tissue distribution, tumor sections were analyzed by imaging mass cytometry (IMC) [9]. Dewaxed and rehydrated FFPE sections were stained with metal-containing antibodies to various structural epithelial and stroma components. Selected areas from each tissue section were then ablated with a deep UV laser focused to a spot size of 1 μm, creating individual plumes of particles from each area. These particles were then transferred with high time fidelity to the Helios™ mass cytometer. Individual isotopes of each metal were simultaneously detected and indexed against the source location, yielding an intensity and spatial map of the distribution of biomarkers, including platinum in tissue. The most abundant isotope of platinum, ^{195}Pt, was used for illustration. Antibody to collagen I, labeled with thulium isotope (^{169}Tm-collagen I), and antibody against alpha-smooth muscle actin, labeled with praseodymium isotope (^{141}Pr-αSMA), were used for staining stromal components.

Trichrome staining was performed using standard pathological technique [10]. Quantification of interstitial collagen content was performed by digital image analysis with HALO™ (Indica Labs, Corrales, New Mexico, USA) by comparing areas occupied by collagen and other tissues. The Ki67 proliferation index was assessed by immunohistochemistry.

For tissue platinum quantification, one 5-μm section of the FFPE tissue was used for each patient. FFPE sections were dewaxed and rehydrated as usual, and then processed and analyzed by high-performance liquid chromatography mass spectrometry (HPLC-MS/MS) system as described previously [11].

Statistics

Due to the limited number of samples, patients were grouped into those with any pathological response (TRG 0–2) and those with no response (TRG 3), for statistical analysis. Student's *t* test was used to compare tissue platinum concentrations and collagen contents between patients with response versus no response. Two-sided $P < 0.05$ was considered to be statistically significant. Platinum concentrations below the lower limit of quantification (30 pg) were treated as 30 pg for analysis.

Results

Tumor tissues were collected from 10 patients, and adjacent normal tissues were available from 7 of the 10 patients. Patient characteristics are listed in Table 1. The median age of patients was 60.5 years (range: 52–76 years). Among these 10 patients, 9 patients received 3 cycles of preoperative

Table 1 Demographic and clinical characteristics of patients

Characteristics	No. of patients ($N = 10$)
Age, years	
Median	60.5
Range	52–76
Sex	
Male	9
Female	1
Tumor site	
GEJ[a]	5
Gastric	5
Pathology stage	
T stage	
ypT3-4a	8
ypT0	2
N stage	
ypN+	4
ypN0	6

[a] Gastroesophageal junction

chemotherapy and 1 received 2 cycles. Three patients received ECF and 7 received ECX. The median cumulative cisplatin dose was 166.8 mg/m^2 (range: 95.9–181.1 mg/m^2), and surgery was performed at a median time of 49 days (range: 28–72 days) after the last cycle of chemotherapy. The cumulative cisplatin dosage, radiologic response, pathological response, collagen content, Ki67 index, and tissue platinum concentrations are shown in Table 2.

Radiological responses did not appear to correspond to pathological responses to preoperative chemotherapy. In three patients with radiological partial response (PR), pathological response ranged from TRG 1 to 3. Similarly, the pathological response ranged from TRG 0 to 3 in seven patients deemed to have radiologically stable disease (SD).

In patients who had any pathological response after preoperative chemotherapy (TRG 0–2), the mean platinum level in tumor tissue was 893 ± 460 pg significantly higher than those in patients with no response (TRG 3), 38.8 ± 17.5 pg ($P = 0.007$) (Fig. 1a). The mean platinum concentration in the adjacent normal tissue was 206.7 ± 81.8 pg. In four patients with TRG 3, platinum concentrations in tumor tissues were lower than those in adjacent normal tissues. However, platinum concentrations were higher in tumor tissues than those in adjacent normal tissues for three patients with TRG 0–2 (Fig. 1b).

The Ki67 index was not detectable in two patients with TRG 0 and 95 and 90% in 2/4 patients with TRG 3. It ranged from 15 to 50% in the rest of patients. The collagen content was significantly higher in patients with TRG 0–2 than in those with TRG 3 (37.4 ± 6.8 vs. 11.5 ± 8.6%, $P < 0.05$) (Fig. 2).

Platinum preferentially localized to collagen fibers. There was extensive binding to collagen fibers in patients who had TRG 0 (Fig. 3a–c), but minimal in patients who had TRG 3 (Fig. 3d–f).

Discussion

The Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) study demonstrated that perioperative chemotherapy improved the overall survival of patients with gastric or GEJ cancers. However, only 41.6% patients assigned to perioperative chemotherapy were able to complete all 3 cycles of scheduled postoperative chemotherapy. Given the difficulties associated with postoperative chemotherapy, an important clinical question is to identify those patients who might benefit from postoperative chemotherapy [2, 3]. Although TRG has not been consistently shown to predict patient survival [5, 6, 12], one recent retrospective review suggested that patients who responded to preoperative chemotherapy seemed to gain a significant survival benefit with postoperative chemotherapy, in contrast to those without responses to preoperative chemotherapy [13]. Furthermore, pathological responses occur in only 40–60% of patients after preoperative chemotherapy. It is not clear why patients exhibit different responses to the same combination of chemotherapeutic agents. We sought to determine whether tumor platinum concentrations could partially explain the interpatient variability in pathological responses.

Tumor platinum concentrations varied widely, from being nondetectable in three out of four patients with no response (TRG 3), to 1694 pg/g in one patient with complete response (TRG 0). They did not correlate with the total dose of cisplatin received or time from last treatment. These findings were similar to those from a previous report in patients with non-small cell lung cancer (NSCLC) [14]. Reduced intracellular platinum concentrations have been identified as a potential explanation of cisplatin resistance, but the mechanisms of this reduction are not clear [15, 16]. Drug transporters, both uptake and efflux transporters, have been implicated [17]. Extracellular matrix and tumor microenvironment play important roles in drug resistance as well. Dense collagen deposition is commonly thought to inhibit drug penetration into tumor tissues. However, the presence of collagen may promote platinum uptake by tumor tissues due to unique platinum properties. Chang et al. previously showed that there was intense binding of platinum to collagen in pancreatic cancer patient-derived xenograft models treated with cisplatin [9]. Our results confirm that platinum binds to collagen in tumor tissues from patients as well. Furthermore, the collagen content was significantly higher in patients with TRG 0–2 than those with TRG 3. It is possible, therefore, that the collagen content in tumor tissues determines the amount of tissue platinum. A

Table 2 Characteristics of treatment, responses, and platinum levels

Patient	Cisplatin dose ($mg/m^{2)}$	Time from last chemotherapy (days)	Radiologic response	TRG[a]	Ki67 (%)	Percent of collagen	Platinum level (pg)	
							Tumor tissue	Adjacent tissue
1	179.35	62	SD[b]	Grade 3	95	8.7	ND[c]	127
2	95.91	36	SD	Grade 3	30	36.6	65	130
3	149.42	72	SD	Grade 2	40	50.6	1092	347
4	110.43	59	SD	Grade 2	15	24.4	364	NA[d]
5	155.56	48	SD	Grade 3	90	0.3	ND	140
6	178.5	28	PR[e]	Grade 2	25	62.8	657	259
7	171.59	55	SD	Grade 0	ND	18.7	657	NA
8	178.13	51	PR	Grade 3	50	0.5	ND	239
9	181.1	44	SD	Grade 0	ND	38.7	1684	NA
10	162.12	40	PR	Grade 1	50	29.3	904	205

[a] Tumor regression grade

[b] Stable disease

[c] Not detectable

[d] Not available

[e] Partial response

higher tissue collagen content is associated with higher tissue platinum concentrations due to platinum binding, hence improved pathological response to cisplatin containing preoperative chemotherapy.

The Ki67 index is a standard marker for cell proliferation. While it was highest in two of four patients with TRG 3 (90 and 95%, respectively), and not detectable in two patients with TRG 0, there was a wide variation in the Ki67 index, indicating that it alone is not a reliable predictor of response.

In contrast to the wide variation in tumor platinum concentrations, platinum concentrations in adjacent normal tissues ranged from 127 to 347 pg. In four patients with TRG 3, tumor platinum concentrations were lower than those in adjacent normal tissues, while tumor platinum concentrations were

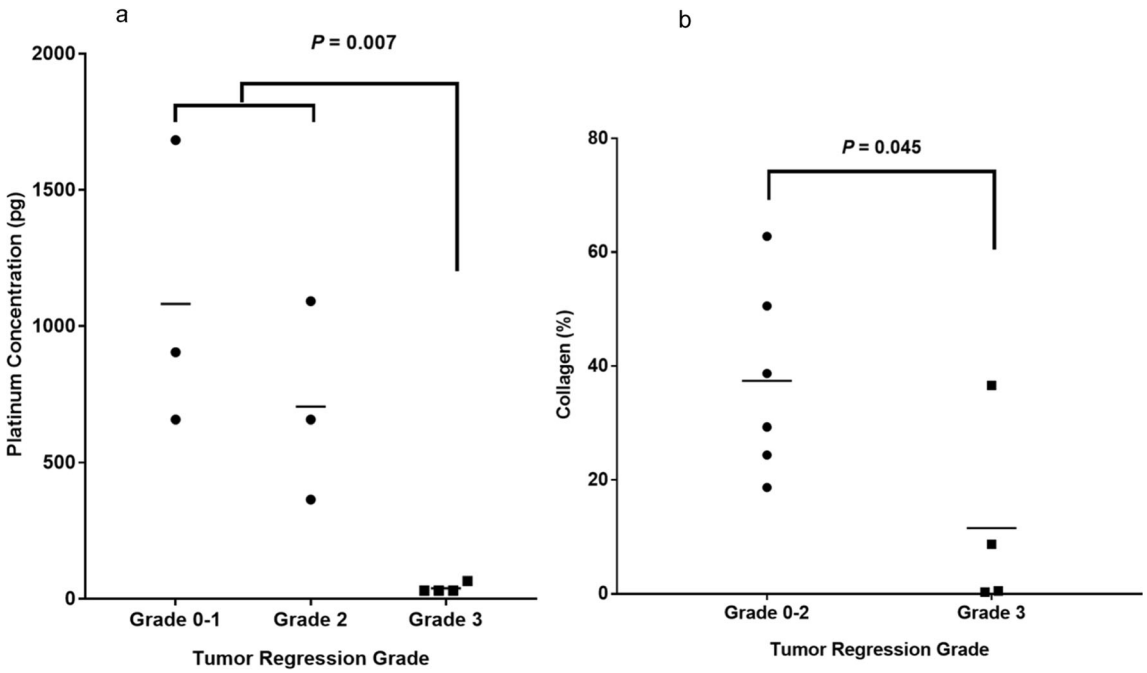

Fig. 1 Platinum concentrations by pathological responses (**a**) and collagen content by pathological responses (**b**)

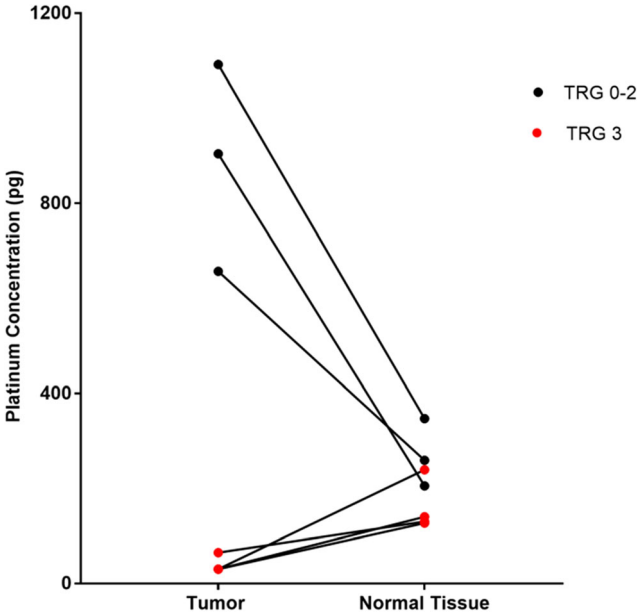

Fig. 2 Comparison of platinum concentrations between tumor and adjacent normal tissues

higher in three patients with TRG 0–2. Due to limited tissue availability, the collagen content in normal tissues was not determined. It was shown previously that the collagen content in pancreatic tissue was approximately 3× higher than that in normal pancreatic tissue [18]. Therefore, it is likely that the collagen content in TRG 0–2 tumors is higher than that in adjacent normal tissues, while the collagen content in TRG 3 tumors is lower. The difference in tumor collagen contents among patients results in varying tissue platinum levels and differential pathological responses to cisplatin-containing chemotherapy.

Tumor platinum concentrations were reported to be associated with response to platinum-containing chemotherapy in patients with NSCLC. In a series of 44 NSCLC patients who were treated with preoperative platinum containing chemotherapy and surgical resection, the percent viable tumor correlated with tissue platinum concentrations. The percent reduction in tumor size based on pre- and post-treatment CT imaging also correlated with tissue platinum concentrations. More importantly, the tissue platinum concentration, not the percent reduction in tumor size, was found to be a significant predictor for time to recurrence, progression-free survival, and overall survival in these patients [14].

Another interesting finding was that CT assessment for tumor response following preoperative chemotherapy did not correspond well with pathological tumor response. CT assessment is known to perform poorly in identifying patients

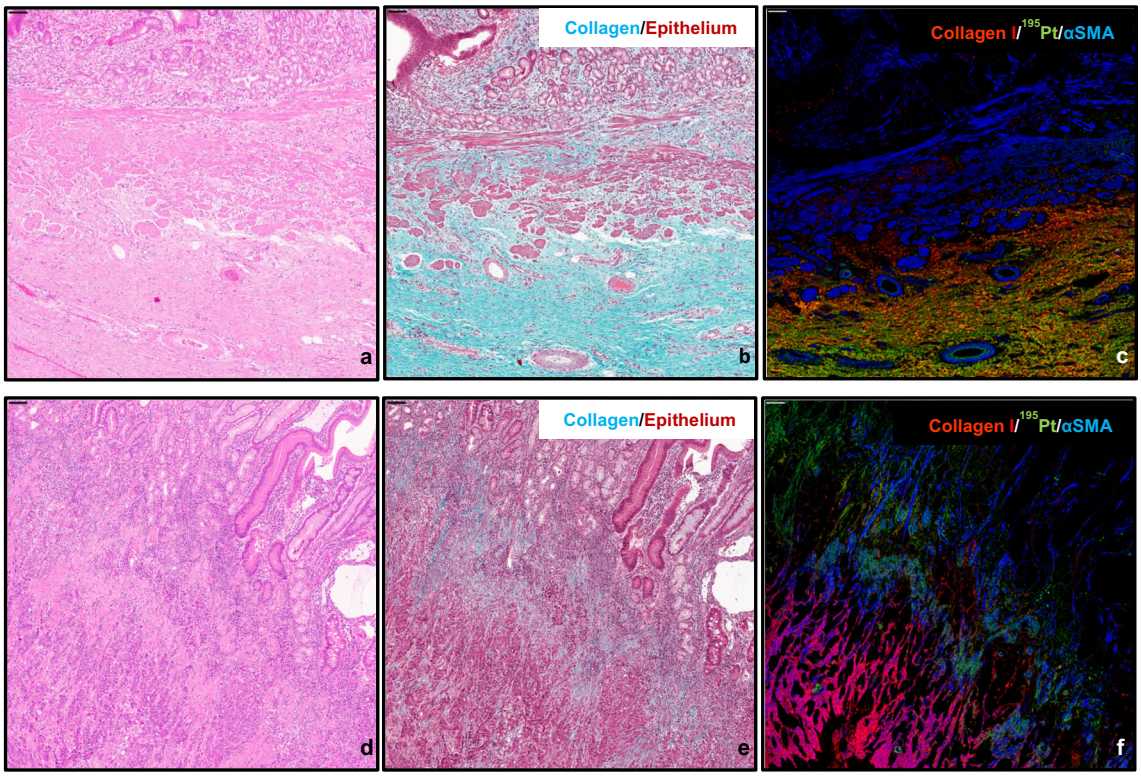

Fig. 3 **a**–**c** Images of a sample with grade 0 response. **a** H&E staining. There is no tumor residual. Scale bar = 100 μm. **b** Masson's trichrome staining (18.7% collagen). Scale bar = 100 μm. **c** IMC image. Platinum colocalized with collagen. Scale bar = 100 μm. **d**–**f** Images of a sample with grade 3 response. **d** H&E staining. There is extensive residual tumor with no definite response. Scale bar = 100 μm. **e** Masson's trichrome staining (0.3% collagen). Scale bar = 100 μm. **f** IMC image. Platinum distribution in the tissue, but not colocalized with collagen. Scale bar = 100 μm

with locally advanced gastric cancer. Park et al. reported that the accuracy of CT was only 57% for T classification and 37% for N classification after chemotherapy with docetaxel and cisplatin [19]. Furthermore, the degree of tumor down staging based on CT assessment did not predict overall survival.

There are several limitations of the present study. First, tumor tissues were available from only 10 patients, and adjacent normal tissues were available from 7 patients. Our findings, although promising, should be considered preliminary. Second, no correlation with clinical outcomes was attempted, given the small sample size and limited statistical power. Third, only the role of platinum was evaluated, although patients were treated with epirubicin and 5-FU/capecitabine as well. We were not able to evaluate the role these two agents played in tumor response to preoperative chemotherapy. Finally, the trichrome stain is specific for type I collagen; therefore, we were not able to evaluate the contribution of other types of collagen to platinum binding. However, type I collagen accounts for a considerable proportion of the total tissue collagen content, and maybe a reasonable proxy of tissue collagen contents.

Despite these limitations, our results are consistent with previous reports. Our findings suggest that tumor platinum concentrations play an important role in determining pathological responses to preoperative chemotherapy in patients with gastric or GEJ cancer. Through a novel imaging technology, we show for the first time in patient tumor tissues that platinum is preferentially localized with collagen, providing a potential explanation for the wide variation in tumor platinum concentrations among patients. Given these promising findings, further studies are warranted to validate these findings and to evaluate whether tumor platinum concentrations are better biomarkers in predicting patient outcomes.

Acknowledgments We thank Jing Xu for valuable technical assistance.

Author's Contributions EC and YC conceived and designed the study and analyzed the data. QC performed IMC analysis. MC analyzed the trichrome and Ki67 data. WZ performed tissue platinum analysis. All authors made substantial contributions towards drafting the manuscript, reviewed the final manuscript for intellectual content, and authorized the submission. All authors read and approved the final manuscript.

Funding This study was funded by the Princess Margaret Cancer Foundation.

Compliance with Ethical Standards

Ethical Standards This study was approved by the Research Ethics Board at the University Health Network, Toronto, Canada and conducted in compliance with relevant Ethics Standards.

Conflict of Interest Qing Chang and Olga Ornatsky are employees of Fluidigm Canada Inc. Olga Ornatsky is one of the co-founders of DVS Sciences Inc. (now part of Fluidigm) that invented, developed and manufactures mass cytometry technologies, including the Helios CyTOF system, the Imaging Mass Cytometer and metal-conjugated reagents.

No other authors declared any potential conflicts of interest.

Abbreviations GEJ, Gastroesophageal junction; ECF, Epirubicin, cisplatin and 5-fluorouracil; HR, Hazard ratio; ECX, Epirubicin, cisplatin and capecitabine; FLOT, Docetaxel, oxaliplatin and 5-fluorouracil; CT, Computer tomography; FFPE, Formalin-fixed, paraffin-embedded; TRG, Tumor regression grade; IMC, Imaging mass cytometry; HPLC-MS/MS, High-performance liquid chromatography mass spectrometry; PR, Partial response; SD, Stable disease; NSCLC, Non-small cell lung cancer; ND, Not detectable; NA, Not available

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