Serpin B3 Is Associated with Poor Survival after Chemotherapy and Is a Potential Novel Predictive Biomarker in Advanced Non–Small-Cell Lung Cancer

Gordon Urquhart, MRCP(UK),† Keith M. Kerr, FRCPath,*‡ Marianne Nicolson, FRCP Edin,† Peh Sun Loo, FRCPath,‡ Ravi Sharma, FRCR,† Raj Shrimali, FRCR,† and Russell D. Petty, PhD MRCP(UK)*†*

Introduction: In a previous biomarker discovery project using gene-expression profiling we identified Serpin B3 (SB3) as a predictor of resistance to platinum doublet chemotherapy (PtC) in non–small-cell lung cancer (NSCLC). This independent prospective study was designed to confirm the predictive utility of SB3.

Methods: SB3 immunohistochemistry was scored by previously validated criteria (score 0 = negative, score 1 = 1%–10% tumor cells positive, score 2 = 11%–50% tumor cells positive, and score 3 = >50% tumor cell positive) in 197 patients with stage IV NSCLC treated with PtC. This provided 80% power to detect a median survival increase from 150 days in patients with an SB3 immunohistochemistry score of 2 or more to 300 days in those with an SB3 score of 0 or 1.

Results: Thirty-six percent of NSCLCs stained positive for SB3. Median survival for SB3 negative/score 0 was 332 days, SB3 positive/score 1 was 268 days, and SB3 positive/score 2 or 3 was 120 days ($p = 0.004$). Cox proportional hazards analysis demonstrated that SB3 positivity is an independent predictor of survival (hazard ratio = 1.87; 95% confidence interval, 1.29–2.71; $p = 0.001$).The disease control rate in SB3 score 0, 1 = 65%, and score of 2 or more = 20 % ($p = 0.002$), with median survival 306 days (score 0, 1) versus 120 days (score ≥2, hazard ratio= 1.71; 95% confidence interval. 1.14–3.10; $p = 0.002$).

Conclusions: SB3-positive immunohistochemistry score of 2 or more (>10% tumor cells positive) identifies a subgroup of patients with stage IV NSCLC who have a poor survival (median 120 days) when treated with PtC, similar to that estimated for untreated or chemo-refractory stage IV NSCLC. Further prospective qualification using biospecimens from randomized studies is needed, but SB3 seems to be a useful biomarker that identifies a highly resistant subgroup in whom PtC should be avoided.

Key Words: Biomarkers, Chemotherapy, Non–small-cell lung cancer, Serpin B3.

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The molecular characterization of non–small-cell lung cancer (NSCLC) has enabled predictive biomarker directed use of targeted therapies.[1] However, for the majority of patients a targeted therapy is not currently available and platinum (PtC) combination chemotherapy remains a key part of the systemic treatment in advanced-stage disease.[2] Considering the toxicity of PtC, the avoidance of ineffective therapy is desirable, particularly in the vulnerable group of patients with advanced NSCLC. Accordingly, preclinical and clinical investigation continues to identify predictive biomarkers for PtC in advanced NSCLC.[3] Putative predictive biomarkers for PtC in NSCLC include nucleotide excision repair components, most notably ERCC1 for cisplatin and carboplatin,[4–6] BRCA1 for cisplatin and carboplatin,[7,8] and RRM1 for gemcitabine,[9,10] class III β-tubulin for taxanes,[7,11,12] p53 for cisplatin/vinorelbine,[13] MSH2 for cisplatin,[14] p27 for cisplatin-based therapy[15] and thymidylate synthetase for pemetrexed.[16] However, despite extensive preclinical investigation and retrospective/prospective clinical investigation in advanced NSCLC, no reliable predictive biomarkers for PtC are recommended for clinical use,[17,18] and there remains a clinical need for effective predictive biomarkers.

Previously we undertook a hypothesis-generating study using gene-expression profiling to discover new predictive biomarkers for PtC in NSCLC.[19] Genes whose expression was significantly and consistently correlated with the response to PtC in patients with advanced NSCLC were identified and the derived gene-expression signature enabled prediction of the response phenotype.[19] Within this signature, expression of the serine protease Serpin B3 demonstrated a strong correlation with response ($p < 0.0001$) so was selected as a lead predictive biomarker for PtC, and retrospectively qualified in an independent set ($n = 36$) of advanced NSCLC using immunohistochemistry (IHC). Characterization of Serpin B3 expression by IHC allowed a simple scoring system to be developed and

*School of Medicine and Dentistry, University of Aberdeen, Foresterhill Healthcare Campus, Foresterhill, Aberdeen, Scotland, United Kingdom; and Departments of †Oncology and ‡Pathology Aberdeen Royal Infirmary, Foresterhill Healthcare Campus, Foresterhill, Aberdeen, Scotland, United Kingdom.

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Address for correspondence: Russell D. Petty, PhD MRCP(UK), Institute of Medical Sciences, School of Medicine, Foresterhill Healthcare Campus, Foresterhill, Aberdeen AB25 2ZN, Scotland, United Kingdom. E-mail: r.d.petty@abdn.ac.uk

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technically validated according to tumor cell positivity (tcp): Score 0=negative, score 1 = 1% to 10% tcp, score 2 = 10% to 50% tcp, and score 3 of 50% or higher tcp.[19] In an independent set of 36 advanced NSCLCs, use of the scoring system revealed a significant correlation between Serpin B3 protein expression and radiological response to PtC.[19] Serpin B3 expression was associated with lack of response and no responses were seen in patients with Serpin B3 IHC scores of 2 or more (≥10% tcp), suggesting that this represented a chemoresistant subgroup and that Serpin B3 IHC could provide a clinically useful predictive biomarker for PtC in advanced NSCLC.

Serpin B3 is a serine protease that also inhibits a number of lysosomal cathepsins.[20,21] The full physiological and pathological function of Serpin B3 is not fully elucidated but it is clear that it has an important role in the prevention of cell and tissue damage from proteolysis.[20,21] Serpin B3 is overexpressed in many human cancers, and preclinical studies in several types of cancer have shown that Serpin B3 inhibits cell death initiated by a wide variety of different stimuli including cytotoxic drugs such as cisplatin and etoposide.[19,22–27] In breast cancer Serpin B3 overexpression is associated with resistance to anthracycline-based neoadjuvant chemotherapy.[28] Serpin B3 also has demonstrated roles in epithelial to mesenchymal transition, prevention of airway damage in chronic inflammatory states and pulmonary fibrosis.[22,29,30] Serpin B3 is expressed in the cytosol and can be detected in the serum of cancer patients because of *passive* overflow from tumor cytosol as a result of overexpression rather than active secretion.[31] Accordingly, a role for Serpin B3 in mediating PtC resistance in NSCLC seems biologically plausible and the aim of the current study was to qualify the predictive utility of Serpin B3 in an adequately powered independent prospective cohort. We hypothesized that high Serpin B3 expression in NSCLCs, defined as more than 10% tumor cells positive in the immunohistochemical assay (IHC score ≥2) would predict PtC resistance and poor survival, potentially providing a clinically useful biomarker by identifying a highly resistant subgroup of stage IV NSCLC patients in whom PtC should be avoided and alternative therapeutic strategies investigated.

PATIENTS AND METHODS

Patients and Treatment

The study proceeded with full ethical approval from the North of Scotland Research Ethics Committee complying with the principles of Good Clinical Practice and fully informed participant consent. Patients were eligible if they had a histological diagnosis of NSCLC (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, or unspecified NSCLC), of stage IV (American Joint Committee on Cancer, 6th edition)[32] after standard computed tomography (CT) of neck, chest, and abdomen (magnetic resonance imaging and/or FDG-positron emission tomography CT permitted in equivocal cases) with adequate bone marrow, renal, and hepatic function for systemic chemotherapy, Eastern Cooperative Oncology Group performance status of 0, 1, or 2, and adequate histological material had to be available for immunohistochemical analysis of Serpin B3. Exclusion criterion was any serious comorbid condition, which in the opinion of the treating physician excluded treatment with platinum combination chemotherapy. All potential patients were reviewed by the multidisciplinary team in the regional cancer center at Aberdeen Royal Infirmary, Scotland, to confirm eligibility. Because preclinical and clinical evidence, and the known biochemical, cellular, and physiological roles of Serpin B3 provide a biologically plausible explanation for its role in mediating resistance to cytotoxic drugs with a variety of different mechanisms of action, we hypothesized a role for Serpin B3 in mediating resistance to platinum-based chemotherapy combining cisplatin with different cytotoxic drugs.[19–31] Therefore, treatment was permitted with one of the following standard platinum combination regimens based on physician choice;

TABLE 1. Clinical and Histopathological Features of Advanced NSCLC Cancer Patients Treated with First-Line Platinum Combination Chemotherapy in This Study (n = 197)

Variable	Number
Age	
Median	64
Range	38–78
Sex	
Male	116
Female	81
Smoker	
Ongoing	187
Never	10
Performance status	
0	45
1	137
2	15
Histology	
Adenocarcinoma	86
Squamous cell carcinoma	62
Large cell carcinoma	30
Unspecified	19
Weight loss	
>10%	75
<10%	122
Platinum agent	
Cisplatin	128
Carboplatin	69
Combination agent	
Docetaxel	62
Gemcitabine	105
Paclitaxel	4
Pemetrexed	18
Vinorelbine	8
Radiotherapy	
Yes	88
No	109
Second-line treatment	
Erlotinib/gefitinib	33
Chemotherapy	25

NSCLC, non–small-cell lung cancer.

TABLE 2. Seprin B3 IHC with Clinical, Histopathological, and Treatment Details of Patients (Fisher's Exact Test)

Variable	Serpin B3 IHC			Serpin B3 IHC Score		
	Negative	Positive	*p*	0 and 1	≥2	*p*
Age						
≥Median (≥64 yr)	66	34		89	11	
<Median (<64 yr)	61	36	0.379	88	9	0.815
Sex						
Male	69	47		105	11	
Female	58	23	0.107	72	9	0.812
Smoking history						
Never smoker	4	0		4	0	
Smoker/ex-smoker	123	70	0.299	173	20	1.000
Histology						
Nonsquamous	97	38		120	15	
Squamous	30	32	0.002*	57	5	0.617
Platinum type						
Cisplatin	79	49		114	14	
Carboplatin	48	21	0.272	63	6	0.613
Second drug in regimen						
Taxane	43	23		60	6	
Nontaxane	84	47	1.000	117	14	0.807
Weight loss at presentation						
>10%	48	27		66	9	
<10%	79	73	1.000	111	11	0.628
WHO Performance status						
2	119	63		164	18	
0.1	8	7	0.404	13	2	0.653
Second-line systemic treatment						
Yes	44	13		50	7	
No	83	57	0.021*	127	13	0.604
T stage						
T1/2	49	26		66	9	
T3/4	78	44	0.879	111	11	0.628
N stage						
Node negative	8	6		13	1	
Node positive	119	64	0.571	164	19	1.00
Liver metastases						
Yes	21	8		29	0	
No	106	62	0.204	148	20	0.049*

*indicates significant *P* value.
IHC, immunohistochemistry; WHO, World Health Organization.

3-weekly cisplatin 75 mg/m^2 (or carboplatin area under curve 5 if contraindications to cisplatin) plus pemetrexed 500 mg/m^2 or gemcitabine 1250 mg/m^2 day 1+8 or docetaxel 75 mg/m^2, or paclitaxel 200 mg/m^2, or vinorelbine 25 mg/m^2/week and cisplatin 100 mg/m^2/d, day 1 every 28 days. Up to four cycles of treatment were permitted with clinical examination, hematological and biochemical laboratory investigations and chest radiograph before each cycle. Dose modifications for toxicity were performed according to protocol and a restaging CT scan was performed after four cycles or earlier if tumor progression was suspected. In addition to progression, reasons to discontinue chemotherapy before four cycles included serious toxicity and patient/physician decision. Overall survival was defined as date of study entry until date of death, and response was assessed by Response Evaluation Criteria in Solid Tumors v1.0.[33] After completion of chemotherapy, patients were followed up every 6 to 12 weeks depending upon clinical condition, with physical examination and further investigations according to clinical need. Palliative radiotherapy or second-line treatment or was permitted excepting retreatment with cisplatin or carboplatin.

Immunohistochemistry

IHC was preformed according to the method previously described in detail and scored as before,[19] using validated

criteria, namely score 0=negative, score 1 = 1% to 10% tcp, score 2 = 10% to 50% tcp, and score 3 of 50% or more tcp, regardless of staining intensity. Cases were scored independently by two histopathologists and agreement was high with κ statistic = 0.92; $p < 0.001$. Scoring discrepancies were resolved by consensus after review of cases at a double-headed microscope. Treating physicians and patients were blind to Serpin B3 score and scorers were blind to the treatment outcomes.

Statistical Analysis

Previously we saw no responses in 36 patients with advanced NSCLC who had a Serpin B3 IHC score of 2 or more. Responses were seen exclusively in those with a score of 0, or 1.[19] Accordingly, we hypothesized that patients with a Serpin B3 IHC score of 2 or more would have chemoresistant tumors and a poor survival. Assuming that 12% of NSCLCs have an IHC score of 2 or more[19] then considering $\alpha = 0.05$, and a median survival for Serpin B3 patients, IHC score of 2 or more of 150 days consistent with their resistance to PtC, and 300 days in Serpin B3 IHC score of 0 and 1, then a cohort of 190 patients would provide a power of 0.80. Fisher's exact test was used for categorical variables and Kaplan–Meier and Cox proportional hazard model for survival analysis and all p values are two-sided. Statistical analysis was performed using SPSS statistics 20.

RESULTS

Clinical Features of Patients

One hundred ninety-seven patients were recruited between January 2008 and May 2010 (Table 1). A participant flow diagram is shown in Supplementary Digital Content 1 (http://links.lww.com/JTO/A485) in accordance with the Recommendations for Tumour Marker Prognostic Studies (REMARK) guidelines. Twenty-nine percent of patients received second-line systemic treatment and 45% palliative radiotherapy (Table 1). Median follow-up for patients still alive was 773 days.

Serpin B3 IHC

Positive Serpin B3 staining was seen in 36% of NSCLCs (70 of 197) in this cohort (25% [$n = 50$] score 1 and 11% [$n = 20$] score 2 or 3) similar to our previous study.[19] Representative examples of Serpin B3 IHC scores are provided in Supplementary Digital Content 2 (http://links.lww.com/JTO/A486). Serpin B3 was more frequently positive in squamous histology ($p < 0.002$), but Serpin B3 IHC score of 2 or more (the prespecified cutoff for treatment outcome prediction) was not more frequent in squamous histology (Table 2). Low (IHC score 1) or negative Serpin B3 was found more frequently in patients with liver metastases (p=0.049; Table 2). Serpin B3 positive patients received second-line therapy less often ($p = 0.021$), but there was no difference in the frequency of second-line chemotherapy in patients with Serpin B3 IHC score of 0 and 1 versus those with 2 or more (the prespecified cutoff for treatment outcome prediction). Serpin B3 IHC was not associated with age, sex, smoking history, performance status, weight loss, or type of chemotherapy treatment(Table 2).

Serpin B3 IHC and Overall Survival

Serpin B3 expression was strongly correlated with overall survival. Median survival according to Serpin B3 was: score 0 (negative)= 332 days, score 1 = 268 days, and score 2

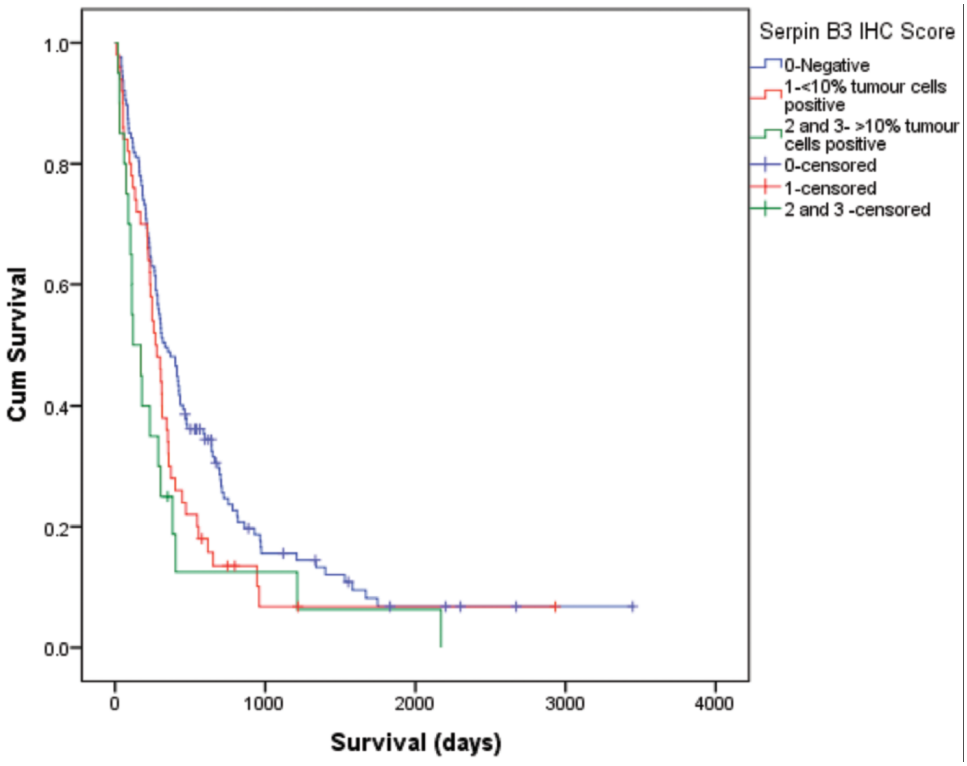

FIGURE 1. Kaplan–Meier survival analysis for Serpin B3 in advanced NSCLC patients (n = 197) treated with platinum combination chemotherapy (Serpin B3 IHC Score 0 = negative, Score 1 = 1–10% tumour cells positive, Score 2 = 10–50% tumour cells positive and Score 3 = >50% tumour cell positive). NSCLC, non–small-cell lung cancer; IHC, immunohistochemistry.

or 3 = 120 days (Fig. 1; log rank $p = 0.004$). Cox proportional hazards analysis demonstrated that Serpin B3 positivity is an independent predictor of survival (Table 3 hazard ratio [HR] = 1.87; 95% confidence interval [CI], 1.29–2.71; $p = 0.001$).

Applying the predefined stratification cutoff of Serpin B3 IHC score of 2 or more,[21] median survival in patients with score 0 or 1 was 306 days and median survival in score of 2 or more was 120 days (Fig. 2, HR= 1.71; 95% CI, 1.14–3.10; $p = 0.002$). Cox proportional hazards analysis demonstrated that Serpin B3 of 2 or more is an independent predictor of survival (Table 4; HR= 1.46; 95% CI, 1.11–2.91; $p = 0.006$).

Serpin B3 and Response to First-Line Platinum-Based Chemotherapy

Applying the predefined stratification cutoff, Serpin B3 IHC score of 2 or more was associated with a worse disease control rate (disease control rate [DCR] = Response Evaluation Criteria in Solid Tumors partial response + complete response + stable disease) to first-line PtC. The DCR in score 0 or 1 = 65%, DCR in score 2or higher= 20%; $p = 0.002$).

TABLE 3. Cox Proportional Hazards Analysis Demonstrating that Serpin B3 Positivity Is an Independent Predictor of Survival

Variable	HR (95% CI)	*p*
Age	0.83	0.245
≥Median	(0.60–1.14)	
Smoking history	0.54	0.254
Never smoker vs. smoker /ex-smoker	(0.19–1.56)	
Histology	1.05	0.801
Squamous vs. nonsquamous	(0.71–1.55)	
Platinum type	0.73	0.085
Cisplatin vs. carboplatin	(0.51–1.05)	
Second drug in regimen	0.90	0.156
Taxane or nontaxane	(0.77–1.04)	
Disease control	0.22	<0.0001*
CR + PR + SD vs. PD	(0.15–0.32)	
Weight loss at presentation	2.09	<0.0001*
>10% vs. <10%	(1.45–2.98)	
WHO performance status	1.47	0.047*
2 vs. 0.1	(1.05–1.99)	
Second-line systemic treatment	0.84	0.360
Yes vs. no	(0.58–1.21)	
T stage	1.05	0.594
T1/2 vs. T3/4	(0.89–1.24)	
N stage	1.22	0.033*
Node positive vs. node negative	(1.02–1.47)	
Liver metastases	1.53	0.001*
Yes vs. no	(1.21–1.93)	
Serpin B3	1.87	0.001*
Positive (IHC score 1, 2, or 3) vs. negative (IHC score 0)	(1.29–2.71)	

*indicates significant *P* value.

HR, hazard ratio; CI, confidence interval; CR, complete response; WHO, World Health Organization; PD, progressive disease; IHC, immunohistochemistry.

DISCUSSION

An improved understanding of the molecular pathogenesis of NSCLC together with the development of targeted therapies has led to the emergence of a molecular classification for the disease where molecular abnormalities in tumors serve as predictive biomarkers to stratify patients for particular therapies.[1] Current qualified predictive biomarkers in NSCLC, namely EGFR mutations and ALK rearrangements identify clinically effective targeted therapies for up to 20% of advanced-stage patients.[34] Newly identified driver mutations or molecular abnormalities amenable to targeting by agents in development may provide new therapeutic opportunities for a further 30% of NSCLCs.[1,34] Therefore, at present, a molecular target/predictive biomarker is not currently available for the majority of patients with NSCLC in whom PtC forms the systemic treatment of choice.[2,17] Accordingly, predictive biomarkers for PtC in NSCLC are required in practice, especially considering the toxicity of chemotherapy and the need to avoid ineffective therapy in this vulnerable group of patients with limited life expectancy. Extensive investigation of predictive biomarkers for chemotherapy in NSCLC has, to date, failed to promote any marker into routine clinical use.[17,18]

Here we present a prospective, adequately powered, independent validation of the impact of Serpin B3 on the survival of advanced NSCLC patients treated with PtC by using a convenient, previously validated IHC assay and scoring system for Serpin B3,[19] and using a hypothesis-driven threshold to define biomarker-positive and -negative patients. In a biomarker discovery investigation we had previously identified that tumors positive for Serpin B3 using IHC tended to be resistant to PtC and also had a poor therapy independent prognosis.[19] NSCLC patients with more than 10% of their tumor cells staining positively for Serpin B3 (IHC score ≥2), were invariably nonresponders.[19] Accordingly, we hypothesized that Serpin B3 could be a clinically useful predictive biomarker to identify patients whose tumors are resistant to PtC.

Our hypothesis is supported by this study and provides independent qualification of the predictive impact of Serpin B3 assayed by IHC in NSCLC. Patients who have stage IV NSCLC with Serpin B3 IHC score of 2 or more (≥ 10% tumor cells positive) are much less likely to achieve disease control (20% versus 65% $p = 0.002$), and have much shorter survival after treatment with PtC (120 days versus 306 days; $p = 0.002$). Serpin B3 positive IHC, that is, any positive IHC staining versus negative, was found more frequently in squamous cell carcinomas, but Serpin B3 IHC score of 2 or more (i.e., >10% tumor cells positive- the prespecified cutoff for treatment outcome prediction) was not more frequent in squamous histology(Table 2). Therefore an interaction between Seprin B3 IHC staining and histological subtype could not have influenced outcome or response to chemotherapy, for example, with pemetrexed, for the patient subgroups stratified by the prespecified and investigated biomarker cutoff of Serpin B3 IHC score of 2 or more. In addition, the multivariate analysis (Table 4) demonstrates that Serpin B3 IHC score of 2 or more is strongly associated with

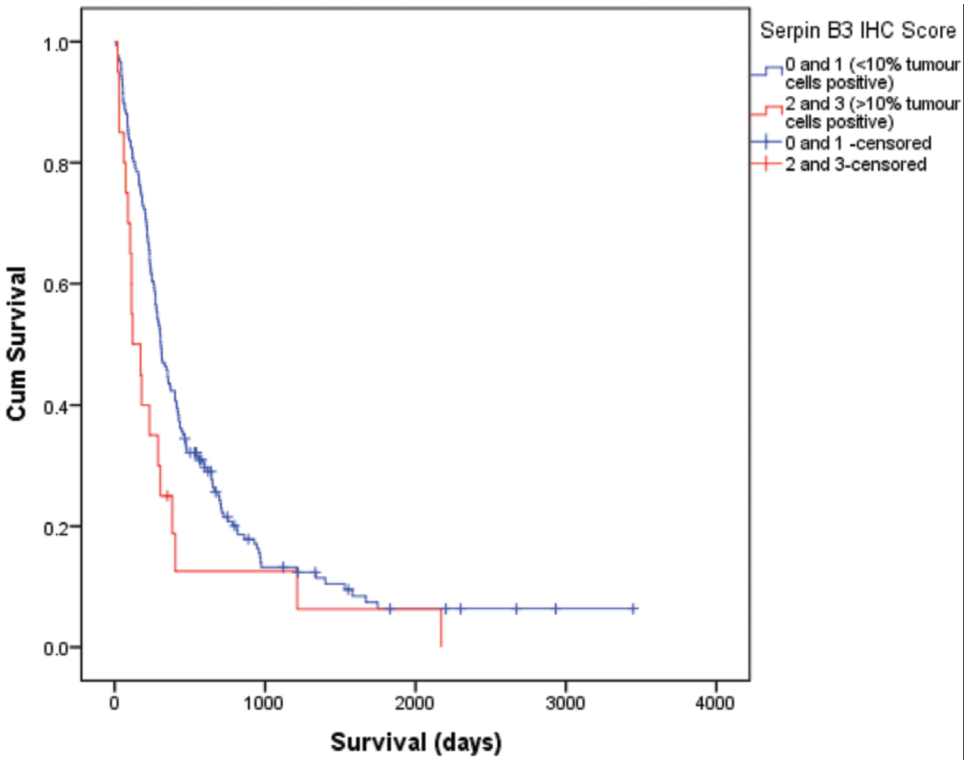

FIGURE 2. Kaplan–Meier survival analysis for Serpin B3 in advanced NSCLC patients (n = 197) treated with platinum combination chemotherapy using predefined Serpin B3 IHC score ≥2 for stratification. NSCLC, non–small-cell lung cancer; IHC, immunohistochemistry.

overall survival independent of histology (squamous versus nonsquamous). Similarly, Serpin B3 positive IHC patients more commonly received second-line therapies, but second-line treatment was not more common in Serpin B3 IHC score of 2 or more. Therefore, the frequency of second-line therapy was not different between the groups of patients stratified by the prespecified biomarker cutoff of Serpin B3 IHC score of 2 or more. Accordingly, differences in frequency of second-line therapy could not have influenced outcome in these Serpin B3 IHC stratified groups. Consistent with this, the multivariate analysis (Table 4) demonstrates that Serpin B3 IHC score of 2 or more is strongly associated with overall survival, independent of whether second-line therapy was given or not.

Serpin B3 IHC score of 2 or more identifies a subgroup of approximately 10% NSCLC patients who have a median survival of only 120 days after treatment with PtC. Clinical experience and data from trials of chemotherapy versus best supportive care suggest that this survival profile of patients with Serpin B3 IHC score of 2 or more is very similar to those treated with supportive care only.[35–39] The multivariate analysis demonstrates that Serpin B3 has utility independent of clinical features that are used by oncologists to determine patient selection for treatment, such as performance status and weight loss. We suggest that Serpin B3 IHC is a useful new predictive biomarker to select patients with advanced NSCLC in whom PtC would be ineffective and who might benefit more by treatment with other therapies. Our study was prospective in terms of patient eligibility, treatment, follow-up, and outcome determination, used a previously validated assay for the biomarker Serpin B3 with a prespecified scoring system with a hypothesis-driven cutoff for assay positivity versus negativity, and was adequately powered. However, without treatment randomization, the predictive versus the prognostic impact of Serpin B3 cannot be definitively determined from this study.[40] We have previously shown that Serpin B3 determined by using the same immunohistochemical assay has therapy-independent prognostic impact in resected NSCLC.[19] The strong correlation between Serpin B3 and response, with a DCR of 65% in Serpin B3 score 0 and 1 (<10% tumor cells positive at IHC), and only 20% in Serpin B3 IHC score of 2 or more (≥10% tumor cells positive at IHC; $p = 0.002$) suggests that the observed difference in survival is predominantly because of a predictive impact for PtC benefit. Evaluation of Serpin B3 in a prospective randomized controlled trial is needed to elucidate the prognostic versus predictive impact and in doing so, define precisely its clinical utility as a biomarker before clinical implementation or a prospective randomized trial where treatment was directed by Serpin B3 IHC as a biomarker could be undertaken. Considering the potential magnitude of the effect on DCRs and survival as observed in our study, there are a number of tumor specimen collections from randomized trials including PtC and other non-PtC treatments arms (some of which are therapeutic trials and others which were designed primarily to determine biomarker utility) that would potentially be suitable and adequately powered to provide *retrospective–prospective* qualification.[3,40] Nevertheless, whether predictive and/or prognostic, the poor survival seen in patients with Serpin B3 IHC score of 2 or more here argues convincingly for the avoidance of PtC in this subgroup of patients. The use

TABLE 4. Cox Proportional Hazards Analysis Demonstrating that Serpin B3 Positivity with Predefined Serpin B3 IHC Score ≥2 for Stratification Is an Independent Predictor of Survival

Variable	HR (95% CI)	*p*
Age	0.82	0.240
≥Median	(0.60–1.14)	
Smoking history	0.64	0.399
Never smoker vs. smoker/ex-smoker	(0.22–1.82)	
Histology	1.21	0.332
Squamous vs. nonsquamous	(0.83–1.77)	
Platinum type	0.78	0.085
Cisplatin vs. carboplatin	(0.54–1.11)	
Second drug in regimen	0.87	0.065
Taxane or nontaxane	(0.75–1.01)	
Disease control	0.23	<0.0001*
CR + PR + SD vs. PD	(0.16–0.33)	
Weight loss at presentation	1.77	0.002*
>10% vs. <10%	(1.23–2.53)	
WHO performance status	1.52	0.019*
2 vs. 0.1	(1.07–2.15)	
Second-line systemic treatment	0.74	0.115
Yes vs. no	(0.51–1.08)	
T stage	1.11	0.228
T1/2 vs. T3/4	(0.94–1.32)	
N stage	1.23	0.026*
Node positive vs. node negative	(1.03–1.48)	
Liver metastases	1.53	0.001*
Yes vs. no	(1.21–1.94)	
Serpin B3	1.46	0.006*
IHC score ≥2 vs. 0 or 1	(1.11–2.91)	

HR, hazard ratio; CI, confidence interval; CR, complete response; WHO, World Health Organization; PD, progressive disease; IHC, immunohistochemistry.

of an IHC assay for Serpin B3 means that it can be readily performed on clinically available formalin-fixed, paraffin-embedded biopsy material and would be feasible as part of a routine clinical histopathology service.

We are performing mechanistic studies using preclinical models to determine the biology of Serpin B3 tumors to provide a rationale for suitable alternate targeted treatment approaches in NSCLC patients with Serpin B3 IHC score of 2 or more. In addition, we are performing mechanistic studies to determine the effect of overexpression of Serpin B3 on sensitivity to individual cytotoxic drugs, although our multivariate analyses suggests multidrug resistance. Our data, including the multivariate analysis, and other preclinical and clinical evidence, as well as the known biochemical, cellular. and physiological roles of Serpin B3,[19–31] suggests a role in mediating resistance to cytotoxic drugs with a variety of different mechanisms of action. This includes resistance to platinum-based chemotherapy combining cisplatin with different cytotoxic drugs in NSCLC, which potentially increases the clinical utility and application of Serpin B3 as a biomarker. Investigation of Serpin B3 expression by IHC in tumor specimens from randomized controlled trials with different PtC regimens and other non-PtC treatments arms is necessary and would also assist in determination of the specific predictive impact of Serpin B3 for different individual cytotoxic drugs.

The identification by Serpin B3 IHC of a subgroup of NSCLC patients who do not benefit from PtC is potentially useful to avoid ineffective and harmful treatment with PtC in these patients. Ideally, alternative effective therapies would be available for Serpin B3 IHC score ≥ 2 patients as part of a biomarker-driven treatment protocol based on Serpin B3 IHC. Further in vitro and in vivo mechanistic investigation of the role of Serpin B3 in NSCLC pathogenesis and response to currently available targeted therapies is required before it would be possible to determine the most appropriate treatments for Serpin B3 IHC score of 2 or more NSCLC patients. Targeting Serpin B3 itself may be feasible, but again this requires further mechanistic investigation.

In conclusion, we have shown that Serpin B3 positive IHC score of 2 (>10% tumor cells positive) identifies a subgroup of patients with stage IV NSCLC who have a poor survival (median 120 days) and low probability of disease control when treated with PtC, which is similar to that estimated for untreated or chemo-refractory stage IV NSCLC. The magnitude of the effect seen suggests that Serpin B3 is a potential clinically useful predictive biomarker that identifies a highly resistant subgroup of advanced NSCLC patients in whom PtC should be avoided and that further prospective qualification in randomized studies is warranted to determine clinical utility.

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