Molecular prediction of adjuvant cisplatin efficacy in Non-Small Cell Lung Cancer (NSCLC)—Validation in two independent cohorts

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Abstract

Introduction

Effective predictive biomarkers for selection of patients benefiting from adjuvant platinum-based chemotherapy in non-small cell lung cancer (NSCLC) are needed. Based on a previously validated methodology, molecular profiles of predicted sensitivity in two patient cohorts are presented.

Methods

The profiles are correlations between in vitro sensitivity to cisplatin and vinorelbine and baseline mRNA expression of the 60 cell lines in the National Cancer Institute panel. An applied clinical samples filter focused the profiles to clinically relevant genes. The profiles were tested on 1) snap-frozen tumors from 133 patients with completely resected stage 1B-2 NSCLC randomized to adjuvant cisplatin and vinorelbine (ACV, n = 71) or no adjuvant treatment (OBS, n = 62) and 2) formalin-fixed paraffin-embedded (FFPE) tumors from 95 patients with completely resected stage 1A-3B NSCLC receiving adjuvant cisplatin and vinorelbine.

Results

The combined cisplatin and vinorelbine profiles showed: 1) univariate Hazard Ratio (HR) for sensitive versus resistant of 0.265 (95% CI:0.079–0.889, p = 0.032) in the ACV cohort and a HR of 0.28 in a multivariate model (95% CI:0.08–1.04, p = 0.0573); 2) significant prediction at 3 year survival from surgery in univariate (HR = 0.138 (95% CI:0.035–0.537), p = 0.004) and multivariate analysis (HR = 0.14 (95% CI:0.030–0.6), p = 0.0081). No discrimination
was found in the OBS cohort ($HR = 1.328$, $p = 0.60$). The cisplatin predictor alone had similar figures with 1) univariate $HR$ of 0.37 (95% CI: 0.12–1.15, $p = 0.09$) in the ACV cohort and 2) univariate $HR$ of 0.14 (95% CI: 0.03–0.59, $p = 0.0076$) to three years. Functional analysis on the cisplatin profile revealed a group of upregulated genes related to RNA splicing as a part of DNA damage repair and apoptosis.

Conclusions
Profiles derived from snap-frozen and FFPE NSCLC tissue were prognostic and predictive in the patients that received cisplatin and vinorelbine but not in the cohort that did not receive adjuvant treatment.

Introduction
Lung cancer account for 1.59 million deaths annually worldwide and 85% of cases are non-small cell lung cancer (NSCLC) [1]. Approximately 30% of NSCLC patients are eligible for surgery [2,3]. Adjuvant platin-based regimens after surgery are standard care in stage 2A-3A with a robust 5% absolute overall survival (OS) benefit. Conversely, patients with stage 1A do not appear to benefit from the adjuvant chemotherapy and results for stage 1B patients are conflicting [4–6]. Since some of the NSCLC patients receiving cisplatin do not benefit from the treatment, given the controversial results for stage 1B patients, and considering that some of stage 1A patients not receiving cisplatin might actually benefit from it, more effective predictive biomarkers for this treatment appear highly warranted.

Several resistance mechanisms have been identified, which all have major impact on the efficacy of cisplatin [7]. The cytotoxicity of cisplatin is attributed to single strand DNA monoadducts, intrastrand crosslinks, and interstrand crosslinks. This DNA damage is either repaired by the DNA damage response (DDR), or leads to apoptosis [8]. DDR relevant to the repair of cisplatin-induced DNA damage includes nucleotide excision repair (NER), interstrand crosslink repair (ICLR/FA), mismatch repair (MMR), homologous recombination (HRe) and non-homologous end joining (NHEJ) [9–12]. Cisplatin resistance is shown to be related to DDR proteins such as upregulation of excision repair cross-complementing 1 (ERCC1) as a part of NER and HRe [13], to secondary mutations in breast cancer 1/2, early onset (BRCA1/2) as a part of HRe [14], and to MutS Homologue 2 (MSH2) as a part of MMR and ICL even if conflicting reports exist [15,16]. Other proposed resistance mechanisms to cisplatin are reduced uptake through copper transporters CTRL and CTRL and increased efflux through pumps such as ATP7A/ATP7B. All of these have been proposed as prognostic biomarkers and as biomarkers predictive to cisplatin efficacy in the early stages of NSCLC, but possibly due to several competing mechanisms of resistance and efficacy of cisplatin no predictive biomarkers to adjuvant chemotherapy are in use in clinic yet [17].

A more comprehensive, highly multivariate model seems to be required to improve precision in treatment decisions. The model used in the present publication utilizes the full transcriptome as the data source, from which a predictive biomarker is developed. The biomarker system is based on a previously validated method with various drugs in various cancers. The basic hypothesis is that patterns of sensitivity and gene expression can translate into clinical efficacy of each tested drug [18–21]. The National Cancer Institute cell line panel of 60 cell lines (NCI60) is the basis of the drug response predictor (DRP) system and the sensitivity patterns of cisplatin and vinorelbine, respectively, were correlated to gene expression of the same
cell lines [22]. This assumes that direct cytotoxic action and other mechanisms of action are not accounted for. The basic profile is then translated into clinical efficacy of each drug by use of additional gene expression data from 3200 tumors of mixed origin.

From the prospective randomized clinical trial JBR.10 Zhu and colleagues made a dataset of 133 stage 1B-2 NSCLC patients with microarray data publicly available [23]. The 133 patients were randomized to receive either adjuvant chemotherapy with cisplatin and vinorelbine (71 patients) or no adjuvant treatment (62 patients) and mRNA was extracted from snap-frozen tumors [24]. We tested the cisplatin marker, the vinorelbine marker and the combined marker on the dataset.

Using archival formalin-fixed paraffin-embedded (FFPE) tissue the profiles were validated in an independent cohort of stage 1A-3B NSCLC patients (RH-cohort) receiving adjuvant chemotherapy with cisplatin and vinorelbine.

Study objectives were to examine the profiles of cisplatin and vinorelbine individually and combined in the two independent datasets, to evaluate reproducibility of the results, and to interpret any predictive value of the markers to identify beneficiaries of adjuvant treatment with cisplatin and vinorelbine.

Materials and methods

Development of in vitro based drug profiles

The model is based on in vitro cytotoxicity for each specific drug tested in the NCI60 cell line panel developed prior to the present study by Medical Prognosis Institute [20]. In this project, correlations of cytotoxicity to cisplatin and vinorelbine, respectively, were combined with the transcriptome of the 60 cell lines in the panel. To maintain only clinically relevant genes, mRNA expression was measured in more than 3200 snap-frozen tumor specimens, and only markers present in clinical tumor material were retained. The final signature consists of two sets of genes, features associated with sensitivity and features associated with resistance. We tested both the mRNA and the miRNA transcriptomes in two separate profiles of each drug. The final gene signature is covered by issued (8,445,198) and pending (62/440,883) patents [25]. To interpret any biological meaningful information we did pathway elucidation on the Affymetrix mRNA U133 cisplatin profile. The probes of the cisplatin profile were annotated to corresponding gene names and submitted to g:Profiler for functional interpretation and presented in S1 Doc are a subset of 73 genes that were positively correlated for association to pathways or gene ontologies and had significantly enriched BIOGRID interactions [26].

JBR.10 dataset

Total RNA from 133 stage 1B-2 NSCLC patients was isolated from snap-frozen tumor samples and hybridized to Affymetrix HG-U133A by Zhu and colleagues. The patients were randomized to receive adjuvant cisplatin and vinorelbine (n = 71; Table 1) or no adjuvant treatment (n = 62) in the JBR.10 randomized clinical trial. This dataset is publicly available on Gene Expression Omnibus as GSE14814 [23].

RH-cohort

To validate the effect of the mRNA profiles in an independent cohort and to facilitate translation to FFPE material that is by far the most clinically used archival tissue source worldwide [27], FFPE tumor tissue and clinical data from patients treated at Copenhagen University Hospital, Rigshospitalet, during the period 2005–2011 was collected. Ninety-five stage 1A-3B NSCLC patients were included.
Included were patients diagnosed with NSCLC receiving adjuvant treatment with at least one full cycle of cisplatin and vinorelbine following complete macroscopic and microscopic resection of tumor. This cohort of primary NSCLCs comprised the common subtypes of primary NSCLC (Table 1 describing overall features and S1 Table describing detailed histological features of the tumors) [28].
Exclusion criteria were neuroendocrine tumor at time of diagnosis, other cancer within 5 years prior to diagnosis (though basal cell carcinoma of skin, spindle cell carcinoma of skin and carcinoma in situ of cervix were eligible), or breast cancer at any time prior to diagnosis. Further patients known to have metastatic disease (M1) at time of adjuvant treatment were excluded even with complete resection of primary tumor. Patients receiving neoadjuvant chemotherapy were not included in the study. All-comers were 169 patients among whom 95 were included with S1 Fig describing the excluded patients.

The medical history of the eligible patients was obtained retrospectively with at least 3.5 years of clinical follow-up for each patient. Clinical covariates tumor stage, tumor histology and treatment dose were determined from patient records and pathological analysis. Staging and histology were assessed by the same certified pathologist on all samples (ESR) and followed the 7th edition TNM-staging recommended by the International Association for the Study of Lung Cancer (IASLC) [29] and the current WHO Classification of tumors of the lung [28], respectively. When necessary histological type (n = 9) and stage (n = 14) were reclassified. The pathologist further evaluated the percentages of tumor cell content (tumor cell nuclei vs. all nuclei in specimen), amount of necrotic tissue, and hemorrhage, as well as possible neuroendocrine features in the examined tissue blocks, as presented in S1 Table.

The microarray data are publicly available on Gene Expression Omnibus as GSE108492.

**Ethics statement**

The JBR.10 cohort is previously published and the original research protocol was approved by the institutional review boards at all the institutions, and all patients provided written informed consent [24].

The RH cohort data collection was approval by the Regional Committee on Health Research Ethics for Capital Region Denmark and in accordance with Declaration of Helsinki. Informed consent was not obtained since the research conducted did not have implications on the health or outcome of the enrolled patients which is in accordance with Danish Law and accepted by the Regional Committee on Health Research Ethics for Capital Region Denmark.

**Study design**

Cases were selected retrospectively based on inclusion and exclusion criteria as presented above. No stratification was done and there were no matched controls. Primary endpoint was overall survival (OS) and secondary endpoint was disease-specific survival (DSS) (NSCLC-specific survival) evaluated by two observers (IKB, JBS). Only when death was very likely not to be caused by NSCLC, the patients were classified as death of other cause. No additional corrections for co-morbidities were done even though many patients had apparent comorbidities (Table 1). Time is calculated from the date of surgery.

Study sample size was set to approximately 100 patients based on statistical power calculations of 96% with a two-sided alpha of 0.05.

**Laboratory analysis (mRNA and miRNA analysis)**

Total RNA was extracted from 5 consecutive 10 μm-thick FFPE sections of tumors resected prior to adjuvant treatment using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE samples (Ambion, Austin, TX, USA) according to the manufacturer’s protocol. Total RNA was extracted from 103 tumor samples from 95 patients, amplified and microRNA run on Affymetrix Genechip® miRNA Array 1.0 (Thermo Fisher Scientific, Waltham, MA, USA) and mRNA on Affymetrix Genechip® Human Genome U133 Plus 2.0 Array and Almac Xcel array (Almac Group, Craigavon BT63 5QD, United Kingdom). When more than one tumor
sample was obtained from the same lobe, the predictions were compared and the sample with the lowest sensitivity score was kept for evaluation.

Normalization of miRNA and mRNA microarray data was performed in R using Robust Multi-array Average (RMA).

The profile scores were defined as the difference between the averages of the two groups (up- and down-regulated) of features for each of the drugs. The scores were then scaled to cover the range from 0 to 100. One cisplatin-sensitivity score, one vinorelbine score, and one combined score were derived for each patient for each platform.

**Statistics**

The association of the drug response predictor (profile) (DRP) marker to the time to the clinical outcomes, disease specific death and death of all causes was assessed using the Cox proportional hazards model on each dataset. Multivariable analysis included age, gender, disease stage and histological type. The DRP marker is scored as a continuous covariate and rescaled so that the hazard ratio (HR) is for a 50-point difference. For each platform, the success criterion is a two-sided p-value of the combined model of less than 0.05.

Model assessment based on martingale residuals demonstrated a significant departure from the proportional hazards assumption in the second dataset (p = 0.013). Therefore, the DRP was entered as a time dependent covariate with a threshold at 3 years. Threshold was defined after the proportional hazards model was rejected but prior to evaluating the method of time dependence.

The two studies were combined using the Cox proportional hazards model with a random effect for study.

Statistical calculations were done using SAS (v9.4, SAS Institute, Cary, N.C., USA) and R (R Development Core Team, Vienna, Austria, http://www.R-project.org).

**Results**

**The profiles**

The Affymetrix U133 Plus 2.0 mRNA profile for cisplatin consisted of 95 probes corresponding to 83 genes correlated to sensitivity and 110 probes corresponding to 100 genes correlated to resistance. The mRNA vinorelbine profile consisted of 52 upregulated probes and 77 downregulated probes (mRNA). The combined marker was a combination of the cisplatin and vinorelbine profiles at gene level and the values were based on all up- and downregulated genes in both profiles. The mRNA based Affymetrix U133 Plus 2.0 combined marker for cisplatin and vinorelbine is considered the primary profile.

Levels of each predictor and rank correlations between the mRNA predictors of cisplatin, vinorelbine and the combined predictor in the RH-cohort are given in S2 Fig. The mRNA-based cisplatin and the mRNA-based combined cisplatin and vinorelbine profiles correlate with a value of 0.87, p < 0.0001. mRNA profiling on Almac Xcel assay was almost indistinguishable from the Affymetrix mRNA U133 Plus 2.0 (correlation coefficient = 0.96 for cisplatin profile), hence we report the Affymetrix mRNA U133 Plus 2.0 results in the following.

The miRNA for cisplatin alone and the combined miRNA cisplatin and vinorelbine correlate poorly to the mRNA markers (p = 0.26 and p = 0.74).

**Baseline demographics and association**

Baseline demographic features of the JBR.10 subpopulation are presented in the original publication by Zhu and colleagues and in Table 1 [23].
Baseline clinical and histological characteristics of the 95 consecutive stage 1A-3B NSCLC patients in the RH-cohort are presented in Table 1. The median time of observation was 76.6 months (reverse Kaplan-Meier method). 43 patients had died with 36 deaths attributed to NSCLC.

The RH-cohort appeared representative of a NSCLC population even with some differences to the JBR.10 cohort [28]. While JBR.10 cohort had 55% adenocarcinoma there were 67% in the RH-cohort and adenocarcinomas had poorer survival than squamous cell carcinomas (p = 0.03). As expected disease stage was a singular prognostic marker (p = 0.02) with outliers in stage 1A that represent too few individuals to be accounted for.

The two cohorts are in general comparable with respect to gender, age and treatments received. There are some differences in regards of the parameters histology and stage. In the RH-cohort, all patients that had radical surgery and ACV from 2005–2011 at Rigshospitalet, Copenhagen, Denmark, were enrolled. Hence included were some outliers in regards of stage (1A and 3B). JBR.10 included only stage 1B-2.

Table 1 shows that there is no association between the combined vinorelbine-cisplatin marker and clinical covariates gender, histology, stage, or age in either datasets (p-values ranges from 0.29–0.83).

Profiles and prognosis

JBR.10-cohort. Kaplan-Meier estimates of disease-specific survival (DSS) of the cohort treated with ACV divided by a score of the combined markers of 50 is presented in Fig 1A. The combined cisplatin and vinorelbine marker profiles in the first cohort scored as a continuous covariate showed a Hazard Ratio (HR) = 0.265 (95% CI:0.079–0.889, p = 0.032) in the ACV cohort (sensitive versus resistant), as shown in Table 2. Similar effect sizes were seen when dichotomizing the profile by the median, HR = 0.52 (95% CI:0.228–1.190, p = 0.12). A multivariate model adjusted for stage demonstrated significance for ACV (HR = 0.284 (95% CI:0.133–0.602, p = 0.002)).

Fig 1. Kaplan-Meier curves of the cohorts receiving adjuvant cisplatin and vinorelbine, disease-specific survival. The curves show the cohort receiving adjuvant chemotherapy (ACV) in JBR.10 (1A) divided by a score of 50 and of the RH-cohort also receiving ACV (1B) divided by a score of 50. Underneath each curve is a description of events and patients at risk at different time points. Red: Combined cisplatin and vinorelbine score > 50, predicted high-likelihood responders to ACV; black: Combined cisplatin and vinorelbine score ≤ 50, predicted low-likelihood responders to ACV.

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CI: 0.086–0.944), p = 0.040) but in a model with stage, sex, 10-year age difference and histology the score only did show a trend (HR = 0.28 (95% CI: 0.08–1.04), p = 0.0573, Table 2). Neither univariate nor multivariate models of the combined markers were statistically significant with overall survival as endpoint (Table 2).

In the control arm that had no adjuvant treatment (OBS) the Kaplan-Meier estimates of DSS divided by a score of the combined markers of 50 is presented in Fig 2. No significant discrimination in the OBS cohort was seen when scoring as a continuous covariate (HR = 1.328 (95% CI: 0.46–3.835), p = 0.60). A multivariate model adjusted for stage confirmed the hazard ratios above and was also not statistically significant (HR = 1.702 (95% CI: 0.575–5.036), p = 0.34).

**RH-cohort.** Kaplan–Meier estimates of DSS of the RH-cohort treated with ACV divided by a score of 50 is presented in Fig 1B.

Due to significant deviation from the proportional hazards assumption in the RH-cohort a time-dependent analysis was warranted. The combined cisplatin and vinorelbine marker profile resulted in a significant prediction for up to 3 years from surgery when using a time-dependent Cox model for a difference of 50 points (Table 2). The HR for 3 years OS was 0.17 (95% CI: 0.05–0.64, p = 0.008) and similarly for DSS the HR was 0.138 (95% CI: 0.035–0.537), p = 0.004 scored as a continuous covariate. HR for 5 years DSS was 0.35 (95% CI: 0.13–1.00, p = 0.050, univariate).

In the time-dependent multivariate model adjusting for stage, gender, 10-year difference of age and histology with a 50-point difference in the score, the HR for 3 years OS was 0.17 (95% CI: 0.04–0.68, p = 0.013) and for DSS HR was 0.14 (95% CI: 0.03–0.60, p = 0.008), as shown in Table 2. In the 5-year multivariate model for DSS HR was 0.40 (95% CI: 0.12–1.28, p = 0.12).

A multivariate model with endpoint DSS adjusting for stage only showed that the predictor remained significant (HR = 0.123 (95% CI: 0.030–0.512), p = 0.004). From three years and beyond, all the estimated HRs were above 1 in the RH-cohort and none were statistically significant.

Additional multivariable analyses using the cisplatin DRP alone revealed similar results as the combined predictor with OS (HR 0.37 (95% CI: 0.19–0.70), p = 0.0024, S2 Table) and DSS (HR 0.37 (95% CI: 0.20–0.72) p = 0.0030, S2 Table) to three years and no effect in the group from three years and beyond.

We also tested a microRNA cisplatin DRP predictor with OS (S3 Table) that revealed no statistically significant prediction at any time and we did not continue with any analysis on the miRNA predictor.

**Pooled analysis of the mRNA data**

A pooled analysis of the two treated cohorts with the combined marker with a 50-point difference for endpoint DSS resulted in a significant prediction (HR = 0.187, (95% CI 0.069–0.508), p = 0.001) up to 3 years from surgery using a random effects model. The entire length of the studies univariate was also significant (HR = 0.34 (95% CI 0.17–0.71), p = 0.0042).

The DRP predicted significantly in the multivariate model with 50-point difference and time-dependent analysis at three years (HR = 0.21 (95% CI 0.07–0.60), p = 0.0036), shown in Table 3. When assessing the effect in the pooled studies from three years and beyond, the effect was not significant (HR = 0.76 (95% CI 0.24–2.45), p = 0.6510). When assessing the effect in the entire length of the studies in a multivariate model of the pooled studies and both markers the estimates were significant (HR 0.35 (95% CI 0.16–0.78), p = 0.0096).

**A model with variable thresholds combined with prognostic features**

To search for a clinical feasible cut-off, we plotted the expected survival in the ACV cohort of JBR.10 in Fig 3 based on the multivariate model in Table 2. The plot shows expected survival
Table 2. Uni- and multivariate model per study, endpoint disease-specific survival and overall survival.

| Model                  | Cohort | Parameter          | Level          | Endpoint Disease-Specific Survival | Overall Survival |
|------------------------|--------|--------------------|----------------|-----------------------------------|-----------------|
|                        |        |                    |                | Hazard Ratio | 95% Confidence Limit for Hazard Ratio | P-value | Hazard Ratio | 95% Confidence Limit for Hazard Ratio | P-value |
| 1: Univariate          | 1      | DRP 50-point       | difference     | 0.26        | (0.08–0.89)  | 0.032 | (0.15–1.15) | 0.09 |
|                        | 2      | DRP 50-point       | difference     | 0.38        | (0.15–0.98) | 0.045 | (0.26–1.42) | 0.25 |
| 2: Univariate Time Dependent | 1      | DRP ≤ 3 years     |                | 0.26        | (0.06–1.16) | 0.08  | 0.26       | (0.07–1.06) | 0.06 |
|                        |        | DRP > 3 years     |                | 0.27        | (0.03–2.21) | 0.22  | 0.75       | (0.16–3.40) | 0.70 |
|                        | 2      | DRP ≤ 3 years     |                | 0.14        | (0.04–0.54) | **0.0042** | 0.17 | (0.05–0.64) | **0.0082** |
|                        |        | DRP > 3 years     |                | 1.08        | (0.28–4.15) | 0.91  | 1.68       | (0.53–5.30) | 0.38 |
| 3: Multivariate model  | 1      | Gender            | Female         | 0.91        | (0.33–2.51) | 0.86  | 0.75       | (0.31–1.80) | 0.52 |
|                        |        | Age 10-year        | difference     | 1.65        | (0.98–2.79) | 0.059 | 1.62       | (1.02–2.59) | **0.042** |
|                        |        | DRP 50-point       | difference     | 0.28        | (0.08–1.04) | 0.057 | 0.45       | (0.16–1.30) | 0.14 |
|                        |        | Histology (vs. AC) | Other          | 1.46        | (0.37–5.68) | 0.59  | 0.88       | (0.24–3.20) | 0.84 |
|                        |        | SCC                |                | 0.40        | (0.14–1.16) | 0.09  | 0.30       | (0.12–0.79) | **0.014** |
|                        |        | Stage (vs. 1)      | 2              | 1.73        | (0.74–4.01) | 0.20  | 1.21       | (0.58–2.53) | 0.62 |
|                        | 2      | Gender            | Female         | 0.87        | (0.42–1.80) | 0.71  | 0.93       | (0.48–1.82) | 0.84 |
|                        |        | Age 10-year        | difference     | 1.50        | (0.96–2.35) | 0.08  | 1.55       | (1.01–2.37) | **0.044** |
|                        |        | DRP 50-point       | difference     | 0.41        | (0.14–1.21) | 0.11  | 0.67       | (0.25–1.78) | 0.42 |
|                        |        | Histology (vs. AC) | Other          | 0.50        | (0.18–1.39) | 0.18  | 0.50       | (0.19–1.27) | 0.14 |
|                        |        | SCC                |                | 0.21        | (0.05–0.93) | **0.040** | 0.35 | (0.12–1.08) | 0.07 |
|                        |        | Stage (vs. 1)      | 2              | 2.45        | (1.04–5.77) | **0.040** | 2.93 | (1.32–6.47) | **0.0080** |
|                        |        | 3                  |                | 2.58        | (1.06–6.29) | **0.037** | 3.44 | (1.47–8.05) | **0.0043** |
| 4: Multivariate Time-dependent analysis per study | 1      | Gender            | Female         | 0.91        | (0.33–2.50) | 0.86  | 0.76       | (0.32–1.84) | 0.55 |
|                        |        | Age 10-year        | difference     | 1.66        | (0.98–2.80) | 0.058 | 1.62       | (1.02–2.58) | **0.042** |
|                        |        | Histology (vs. AC) | Other          | 1.46        | (0.37–5.67) | 0.59  | 0.89       | (0.24–3.28) | 0.86 |
|                        |        | SCC                |                | 0.40        | (0.14–1.15) | 0.09  | 0.31       | (0.12–0.82) | **0.018** |
|                        |        | Stage (vs. 1)      | 2              | 1.73        | (0.75–4.02) | 0.20  | 1.20       | (0.57–2.52) | 0.64 |
|                        |        | DRP ≤ 3 years     |                | 0.31        | (0.07–1.48) | 0.14  | 0.32       | (0.08–1.35) | 0.12 |
|                        |        | DRP > 3 years     |                | 0.22        | (0.02–2.29) | 0.21  | 0.70       | (0.14–3.41) | 0.66 |
|                        | 2      | Gender            | Female         | 0.92        | (0.44–1.90) | 0.81  | 0.99       | (0.50–1.94) | 0.97 |
|                        |        | Age 10-year        | difference     | 1.50        | (0.95–2.37) | 0.08  | 1.53       | (1.00–2.36) | 0.052 |
|                        |        | Histology (vs. AC) | Other          | 0.52        | (0.19–1.44) | 0.21  | 0.53       | (0.21–1.35) | 0.18 |
|                        |        | SCC                |                | 0.21        | (0.05–0.94) | **0.042** | 0.36 | (0.12–1.11) | 0.07 |
|                        |        | Stage (vs. 1)      | 2              | 2.48        | (1.07–5.74) | **0.035** | 2.98 | (1.36–6.49) | **0.0061** |
|                        |        | 3                  |                | 2.61        | (1.07–6.35) | **0.034** | 3.49 | (1.50–8.13) | **0.0038** |
|                        |        | DRP ≤ 3 years     |                | 0.15        | (0.03–0.63) | **0.010** | 0.17 | (0.04–0.72) | **0.016** |
|                        |        | DRP > 3 years     |                | 1.37        | (0.29–6.42) | 0.69  | 2.24       | (0.60–8.37) | 0.23 |

Part 1 represent a univariate model per cohort, part 3 represent a multivariate model per cohort. Parts 2 and 4 represent the time-dependent analysis conducted on each cohort in a univariate and multivariate model respectively. All hazard ratios for DRP are based on a continuous score with a 50-point difference. Cohort 1 refers to the JBR.10 cohort treated with cisplatin and vinorelbine and cohort 2 refers to the RH-cohort. Adenosquamous cell carcinoma is included in the group Other in the analysis.

Abbreviations: AC = adenocarcinoma; ACV = adjuvant cisplatin and vinorelbine; DRP = drug response predictor (profile), the combined cisplatin and vinorelbine predictor; Other = pleomorphic, spindle cell, high grade mucoepidermoid carcinoma and adenosquamous cell carcinoma; SCC = squamous cell carcinoma.

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curves for values of the combined profiles at value levels 10, 25, 50, 75, 90 in a linear model with gender set as male, age set as 62 years (median in that cohort), histology as adenocarcinoma and stage as 1 (Fig 3A) and 2 (Fig 3B), respectively.

**Analysis of the cisplatin profile genes’ functional interrelationship**

The genes of the cisplatin profile genes were submitted to g:Profiler and of the 83 genes in the sensitivity profile, 38 genes had significantly enriched BIOGRID interactions and were thus biologically correlated. Similarly, of the 100 genes in the resistance profile, 35 were had enriched BIOGRID interactions (S1 Doc) [26].

Of those genes some revealed patterns of interest in relation to cisplatin and known biochemistry related to cisplatin efficacy and resistance and the following section describes such findings in the profiles.
The mechanism of action of cisplatin relies on DNA damage which ultimately gives rise to apoptosis. Conversely, the DNA damage response (DDR) of cells may impair the efficacy of the drug by removing and repairing the cisplatin-mediated DNA lesions. Further alternative splicing of some proteins can switch them from anti-apoptotic to pro-apoptotic function as with the example of BCL-X\textsubscript{L} (also present in the profile) \cite{30,31}.

None of the canonical DDR proteins appear in the profiles. However, several proteins, originally associated with RNA editing including alternative splicing, such as SFPQ, SYNCRIP, DICER1, SRSF7, snRNP70, QKI do appear in the sensitivity profile. Recent research has shown that these proteins also participate in repair of double strand DNA breaks, which can be caused by cisplatin \cite{32–36}. Moreover, IFI16 is a BRCA1 binding partner and interacts with p53 and is upregulated in the sensitivity profile \cite{37}.

Both CD93 and Moesin (MSN) are involved in apoptosis and retained in the sensitivity profile in line with theory \cite{38} and ANP32E that removes histone H2A.Z, a splice variant of a histone, is involved in several cancer types \cite{39}.

In the resistance profile BCL2L1 coding for the protein BCL-X\textsubscript{L} is a predictor of cisplatin resistance in ovarian cancer \cite{40,41}. Glutathione peroxidase 2 (GPX2) is upregulated by NR2F2 which is also in the resistance profile \cite{42}. Loss of keratin 8 and 18 (KRT8, KRT18), both present in the resistance profile, has been shown in epithelial cancer cells to increase cisplatin sensitivity and cell migration and are known factors in epithelial-mesenchymal transition \cite{43}.

Overall, we found that potential relevant pathways in prediction of cisplatin efficacy are genes that relate to RNA editing possibly as playing a role in repairing DNA double-strand breaks, but also there are some implications of a connection to both apoptosis in the sensitivity profile and epidermal-mesenchymal transition in the resistance profile.

### Discussion

With the emergence of new effective therapies in advanced stages of NSCLC personalizing treatment by biomarker-guidance has become more relevant. This is also relevant in the adjuvant setting as alternative treatments to cisplatin may appear in the nearer future.

In the current study, two sets of up-and downregulated genes reflecting sensitivity/ resistance to cisplatin and vinorelbine, respectively, were tested in two independent cohorts of
NSCLC patients in the adjuvant setting. This method of identification of predictive biomarker profiles has been applied previously in various cancers with various drugs, e.g. adjuvant 5FU in colorectal cancer and fulvestrant in breast cancer [20,21].
The basic hypothesis of the biomarker system is that *in vitro* sensitivity can translate into clinical utility through an algorithm based on more than 3200 tumors to sort away genes and pathways only expressed *in vitro*. The system has also been externally validated by statisticians at MD Anderson that received blinded predictions and evaluated the accuracy to outcome [18].

In this study, the most important results are the consistent effects of the combined markers of cisplatin and vinorelbine through two independent treated cohorts. Further, the cisplatin marker behaves consistently alone and combined with the vinorelbine marker in the two cohorts. The fact that the markers did not predict an effect in the population that had only surgery emphasizes that the markers are predictive of adjuvant cisplatin and vinorelbine efficacy and not merely prognostic.

Focus should also be put on the stability of the marker system through NSCLC tissue state, since the markers were tested initially on mRNA from snap-frozen tissue in the JBR.10 cohort and then validated on mRNA from FFPE tissue in the RH-cohort. In this respect, FFPE tissue notoriously contains mRNA of poorer quality than the one obtainable from snap-frozen tissue, but on the other hand it is by far the most used material for diagnostic and predictive purposes in the clinical setting [27]. Thus, the robustness of the markers’ validation on FFPE tumor tissue indeed highlights the potential clinical utility of the DRP.

With promising results from ovarian cancer we also tested a microRNA predictor of cisplatin on the RH-cohort [44], however this did not result in any relevant prediction in the current study.

In the RH-cohort we conducted a time-dependent analysis with a cut-off of 3 years as the primary analysis. Follow-up time was at least 3.5 years and therefore a cut-off of 3 years seems reasonable. Most studies consider 5 year survival time and hence we presented those as well. The effect of the biomarker in the RH-cohort is by far the largest before 3 years. The effect weakens with time and at 5 years is just on the 0.05 cut-off of p-value, univariately. Beyond a 5 year cut-off the effect is not visible neither in OS nor DSS. The expectation must though be a pronounced importance of the biomarkers in the beginning of the treatment course. This could be reflecting the increased lung cancer-independent mortality described in lung cancer cohorts as the patients in the RH-cohort are multimorbid patients (Table 1) [45]. Smoking status was not accounted for in the patient records and smoking could be a significant variable influencing outcomes. One could hypothesize that the failure of the DRP to predict a benefit beyond 3 years is related to the development of independent primary tumors due to the mutagenic field effect of tobacco smoke exposure.

Multigene setups are appealing when no other known effective single-hit biomarkers exist, which is the case for most anticancer drugs. Similar to our group, other groups have developed multigene signatures for prediction of treatment efficacy in NSCLC, but still none have entered into clinical practice [23,46–48]. However, we believe multigene profiles will be included in future decision processes as seen *e.g.* in the prognostication of breast cancer guiding patients to adjuvant therapy [49].

Furthermore, the DRP system could be regarded as hypothesis-generating, as genes and pathways involved in cisplatin efficacy and resistance were not all identified previously.

In the cisplatin profile, genes related to RNA editing were abundant and with recent studies pointing to a role in repairing DNA double-strand breaks. Furthermore, there are some implications of a connection to both apoptosis in the sensitivity profile and epithelial-mesenchymal transition in the resistance profile, whereas neither ERCC1, BRCA1 nor MSH2 appeared in the profiles. This could point to new paths of research on cisplatin efficacy and resistance.

**A combined model**

Based on simple marker threshold values from the current study, a prospective phase 2 biomarker-guided study with the cisplatin marker as a companion diagnostic has started. It is
enrolling heavily-pretreated breast cancer patients above a specific score to be treated with liposomal cisplatin [50,51]. But could further elaboration on the model for instance combined with known prognostic factors refine the system? We propose a model as seen in Fig 3 with a continuous score combined with known prognostic factors such as stage and histology. The predicted model in Fig 3 could work as a tool to open a transparent discussion with the patient in an evaluation of expected benefit against toxicity and comorbidity. This could enroll all stage 1 patients with high profile levels (e.g. cutoff above 30%) to treatment with cisplatin. Or it could eventually exclude patients that are not expected to benefit such as frail stage 2 patients in the lower quarter level of the profile. This system could support the open conversation between physician and patient of benefits versus toxicities to treatment.

Conclusions

Multigene biomarkers of cisplatin and vinorelbine robustly identified benefactors of treatment with cisplatin and vinorelbine through two independent cohorts and in both snap-frozen and FFPE tissue, which emphasizes their potential clinical applicability. Since the markers showed no prognostic ability in the observational cohort, they appear to be predicting actual treatment benefit. Biomarkers of drug efficacy are very much called for with anticipated alternate treatment options to cisplatin in all stages of NSCLC. And the current biomarker system could already be useful today as a decision-making tool in stage 1A and 1B where there are uncertainties regarding clinical benefit of cisplatin. Similarly, we see this as a decision-making tool with frail patients in later stages to support any treatment decision.

Supporting information

S1 Doc. Sensitivity and resistance genes in the cisplatin profile from the g:profiles pathway analysis.

S1 Fig. Overview of excluded patients in the RH-cohort. All comers were 169 patients. 74 patients were excluded due to various reasons as described in the figure.

S2 Fig. Levels and correlations between mRNA profiles in the RH-cohort. Presented on the diagonal in the figure is the distribution of the Affymetrix U133 Plus 2.0 mRNA (normalized) cisplatin score, vinorelbine score and computed combined score in the RH-cohort. The panels in the lower left part of the figure show scatter plots of two scores at a time, while the numbers in the upper right panels show the corresponding Spearman correlation coefficients and the p-values for the correlation coefficient.

S1 Table. Histological features of tumors from the RH-cohort. Abbreviations: AC = adenocarcinoma, ACV = adjuvant chemotherapy, ASCC = adenosquamous cell carcinoma, SCC = squamous cell carcinoma.

S2 Table. U133 CIS predictor, endpoint DSS and OS. Part 1 represent a univariate model per cohort, part 3 represent a multivariate model per cohort. Parts 2 and 4 represent the time-dependent analysis conducted on each cohort in a uni- and multivariate model respectively. All hazard ratios for DRP are based on a continuous score with a 50-point difference. Cohort 1 refers to the JBR.10 cohort treated with cisplatin and vinorelbine and cohort 2 refers to the RH-cohort. Adenosquamous cell carcinoma is included in the group Other in the analysis.
Abbreviations: AC = adenocarcinoma; ACV = adjuvant cisplatin and vinorelbine; DRP = drug response predictor (profile), the combined cisplatin and vinorelbine predictor; Other = pleomorphic, spindle cell, high grade mucoepidermoid carcinoma and adenosquamous cell carcinoma; SCC = squamous carcinoma.

S3 Table. Mir CIS predictor, endpoint OS. Multivariate analysis of the miRNA cisplatin predictor in a time-dependent model with a cut-off of 3 years. The predictor is scored as a continuous variable and the hazard ratio estimates are for a 50-point difference. Adenosquamous cell carcinoma is included in the group Other in the analysis. Abbreviations: AC = adenocarcinoma; Other = pleomorphic, spindle cell, high grade mucoepidermoid carcinoma and adenosquamous cell carcinomas; SCC = squamous carcinoma.

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References
1. World Health Organization. International Agency for Research on Cancer. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Lung Cancer [Internet]. 2012 [cited 9 Nov 2016]. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx?cancer=lung
2. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012; 62: 220–241. https://doi.org/10.3322/caac.21149 PMID: 22700443

3. Yoon SM, Shaikh T, Hallman M. Therapeutic management options for stage III non-small cell lung cancer. World J Clin Oncol. Baishideng Publishing Group Inc; 2017; 8: 1–20. https://doi.org/10.5306/wjco.v8i1.1 PMID: 28246882

4. Burdett S, Pignon JP, Tierney J, Tribodet H, Stewart L, Le Pechoux C, et al. Adjuvant chemotherapy for resected early-stage non-small cell lung cancer. Cochrane database Syst Rev. 2015; CD011430. https://doi.org/10.1002/14651858.CD011430 PMID: 25730344

5. Pignon J-P, Tribodet H, Scagliotti G V, Douillard J-Y, Shepherd FA, Stephens RJ, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol. 2008; 26: 3552–9. https://doi.org/10.1200/JCO.2007.13.9030 PMID: 18506026

6. Eberhardt WEE, De Ruyscher D, Weder W, Le Pechoux C, De Leyn P, Hoffmann H, et al. 2nd ESMO Consensus Conference in Lung Cancer: locally advanced stage III non-small-cell lung cancer. Ann Oncol. Oxford University Press; 2015; 26: 1573–88. https://doi.org/10.1093/annonc/mdv187 PMID: 25897013

7. Shen D-W, Pouliot LM, Hall MD, Gottesman MM. Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. Pharmacol Rev. American Society for Pharmacology and Experimental Therapeutics; 2012; 64: 706–21. https://doi.org/10.1124/pr.111.006637 PMID: 22659329

8. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. Nature Publishing Group; 2003; 22: 7265–7279. https://doi.org/10.1038/sj.onc.1206933 PMID: 14576837

9. Torgovnick A, Schumacher B. DNA repair mechanisms in cancer development and therapy. Front Genet. Frontiers; 2015; 6: 157. https://doi.org/10.3389/fgene.2015.00157 PMID: 25954303

10. Kotheapdani A, Patrick SM. Evidence for base excision repair processing of DNA interstrand cross-links. Mutat Res. NIH Public Access; 2013; 743–744: 44–52. https://doi.org/10.1016/j.mrfmmm.2012.11.007 PMID: 23219605

11. Sears CR, Turchi JJ. Complex cisplatin-double strand break (DSB) lesions directly impair cellular non-homologous end-joining (NHEJ) independent of downstream damage response (DDR) pathways. J Biol Chem. American Society for Biochemistry and Molecular Biology; 2012; 287: 24263–72. https://doi.org/10.1074/jbc.M112.3449111 PMID: 22621925

12. Birkelbach M, Ferriolino N, Gheorghiu L, Pläffle HN, Daly B, Ebright MI, et al. Detection of Impaired Homologous Recombination Repair in NSCLC Cells and Tissues. J Thorac Oncol. Waltham Academic Press, Elsevier; 2013; 8: 279–286. https://doi.org/10.1097/JTO.0b013e31827ef83 PMID: 23399959

13. Olaussen KA, Dunant A, Fouret P, Brambilla E, André F, Haddad V, et al. DNA Repair by ERCC1 in Non–Small-Cell Lung Cancer and Cisplatin-Based Adjuvant Chemotherapy. N Engl J Med. 2006; 355: 983–991. https://doi.org/10.1056/NEJMoa060570 PMID: 16957145

14. Dhillon KK, Swisher EM, Taniguchi T. Secondary mutations of BRCA1/2 and drug resistance. Cancer Sci. NIH Public Access; 2011; 102: 663–9. https://doi.org/10.1111/j.1349-7006.2010.01840.x PMID: 21205087

15. Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, Castedo M, et al. Systems biology of cisplatin resistance: past, present and future. Cell Death Dis. 2014; 5: 428. https://doi.org/10.1038/cddis.2014.28

16. Kamal NS, Soria J-C, Mendiboure J, Planchard D, Olaussen KA, Rousseau V, et al. MutS Homologue 2 and the Long-term Benefit of Adjuvant Chemotherapy in Lung Cancer. Clin Cancer Res. 2010; 16: 1206–1215. https://doi.org/10.1158/1078-0432.CCR-09-2204 PMID: 20145178

17. Wallerek S, Sørensen JB. Biomarkers for efficacy of adjuvant chemotherapy following complete resection in NSCLC stages I-IIIA. Eur Respir Rev. 2015; 24: 340–55. https://doi.org/10.1183/16000617.0005814 PMID: 26028645

18. Wang W, Baggerly K a., Knudsen S, Askaa J, Mazin W, Coombes KR. Independent validation of a model using cell line chemosensitivity to predict response to therapy. J Natl Cancer Inst. 2013; 105: 1284–1291. https://doi.org/10.1093/jnci/djt202 PMID: 23964133

19. Chen J-J, Knudsen S, Mazin W, Dahlgaard J, Zhang B. A 71-Gene Signature of TRAIL Sensitivity in Cancer Cells. Mol Cancer Ther. 2012; 11: 34–44. https://doi.org/10.1158/1535-7163.MCT-11-0620 PMID: 22027696

20. Knudsen S, Jensen T, Hansen A, Mazin W, Lindemann J, Kuter I, et al. Development and validation of a gene expression score that predicts response to fulvestrant in breast cancer patients. PLoS One. 2014; 9. https://doi.org/10.1371/journal.pone.0087415 PMID: 24505287
21. Buhl IK, Gerstl S, Delorenzi M, Jensen T, Jensen PB, Bosman F, et al. Cell line derived 5-FU and irinotecan drug-sensitivity profiles evaluated in adjuvant colon cancer trial data. PLoS One. 2016; 11: e0155123. https://doi.org/10.1371/journal.pone.0155123 PMID: 27171152

22. Shoemaker RH. The NC160 human tumour cell line anticancer drug screen. Nat Rev Cancer. 2006; 6: 813–823. https://doi.org/10.1038/nrc1951 PMID: 16990858

23. Zhu C-Q, Ding K, Strumpf D, Weir BA, Meyerson M, Pennell N, et al. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. J Clin Oncol. 2010; 28: 4417–24. https://doi.org/10.1200/JCO.2009.26.4325 PMID: 20823422

24. Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, et al. Vinorelbine plus Cisplatin vs. Docetaxel for Advanced Non-Small Cell Lung Cancer. J Clin Oncol. American Society of Clinical Oncology; 2005; 23: 2589–2597. https://doi.org/10.1200/JCO.2004.10.0155123 PMID: 15972865

25. Knudsen S. Methods, kits and devices for identifying biomarkers of treatment response and use thereof to predict treatment efficacy. Steen Knudsen; US8445198 B2, 2008.

26. Reimand J, Arak T, Adler P, Kolberg L, Reisberg S, Peterson H, et al. g:Profiler—a web server for functional interpretation of gene lists (2016 update). Nucleic Acids Res. Oxford University Press; 2016; 44: W83–9. https://doi.org/10.1093/nar/gkw199 PMID: 27098042

27. Klopfleisch R, Weiss ATA, Gruber AD. Excavation of a buried treasure—DNA, mRNA, miRNA and protein analysis in formalin fixed, paraffin embedded tissues. Histol Histopathol. 2011; 26: 797–810. Available: http://www.ncbi.nlm.nih.gov/pubmed/21472693 https://doi.org/10.14670/HH-26.797 PMID: 21472693

28. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Introduction to The 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus, and Heart. Journal of Thoracic Oncology. 2015. pp. 1240–1242. https://doi.org/10.1097/JTO.0000000000000663 PMID: 26291007

29. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Grooteman PA, Rami-Porta R, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol. 2007; 2: 706–14. https://doi.org/10.1097/JTO.0b013e31812931a PMID: 17762336

30. Shkreta L, Michelle L, Toutant J, Tremblay ML, Chabot B. The DNA damage response pathway regulates the alternative splicing of the apoptotic mediator Bcl-x. J Biol Chem. American Society for Biochemistry and Molecular Biology; 2011; 286: 331–40. https://doi.org/10.1074/jbc.M110.162644 PMID: 20980256

31. Schwert C, Schulze-Osthoff K. Regulation of Apoptosis by Alternative Pre-mRNA Splicing. Mol Cell. 2005; 19: 1–13. https://doi.org/10.1016/j.molcel.2005.05.026 PMID: 15989960

32. Jaafar L, Li Z, Li S, Dynan WS. SFPQ•N ONO and XLF function separately and together to promote DNA double-strand break repair via canonical nonhomologous end joining. Nucleic Acids Res. Oxford University Press; 2017; 45: 1848–1859. https://doi.org/10.1093/nar/gkw1209 PMID: 27924002

33. Yang X, Zou P, Yao J, Yun D, Bao H, Du R, et al. Proteomic dissection of cell type-specific H2AX-interacting protein complex associated with hepatocellular carcinoma. J Proteome Res. NIH Public Access; 2010; 9: 1402–15. https://doi.org/10.1021/pr900932y PMID: 20000738

34. Francia S, Michelin F, Saxena A, Tang D, de Hoon M, Anelli V, et al. Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature. Europe PMC Funders; 2012; 488: 231–5. https://doi.org/10.1038/nature12922 PMID: 24463511

35. Zhang M, Bohlson SS, Dy M, Tenner AJ. Modulated interaction of the ERM protein, moesin, with CD93. Immunology. Wiley-Blackwell; 2011; 115: 63–73. https://doi.org/10.1111/j.1365-2567.2005.02120.x PMID: 15819698

36. Obri A, Ouararhni K, Papin C, Diebold M-L, Padmanabhan K, Marek M, et al. ANP32E is a histone chaperone that removes H2A.Z from chromatin. Nature. 2014; 505: 648–653. https://doi.org/10.1038/nature12922 PMID: 24463911
40. Yu W, Jin C, Lou X, Han X, Li L, He Y, et al. Global Analysis of DNA Methylation by Methyl-Capture Sequencing Reveals Epigenetic Control of Cisplatin Resistance in Ovarian Cancer Cell. Pellegrini M, editor. PLoS One. Public Library of Science; 2011; 6: e29450. https://doi.org/10.1371/journal.pone.0029450 PMID: 22216282

41. Williams J, Lucas PC, Griffith KA, Choi M, Fogoros S, Hu YY, et al. Expression of Bcl-xL in ovarian carcinoma is associated with chemoresistance and recurrent disease. Gynecol Oncol. Blackwell, Oxford, UK; 2005; 96: 287–95. https://doi.org/10.1016/j.ygyno.2004.10.026 PMID: 15661210

42. Singh A, Rangasamy T, Thimmulappa RK, Lee H, Osburn WO, Brigelius-Flohé R, et al. Glutathione peroxidase 2, the major cigarette smoke-inducible isofrom of GPX in lungs, is regulated by Nrf2. Am J Respir Cell Mol Biol. American Thoracic Society; 2006; 35: 639–50. https://doi.org/10.1165/rcmb.2005-0325OC PMID: 16794261

43. Fortier A-M, Asselin E, Cadrim M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 up-regulation. J Biol Chem. American Society for Biochemistry and Molecular Biology; 2013; 288: 11555–71. https://doi.org/10.1074/jbc.M112.428920 PMID: 23449973

44. Prahm KP, Høgdall C, Karløsen MA, Christensen IJ, Novotny GW, Knudsen S, et al. Clinical validation of chemotherapy predictors developed on global microRNA expression in the NCI60 cell line panel tested in ovarian cancer. Lee JW, editor. PLoS One. Public Library of Science; 2017; 12: e0174300. https://doi.org/10.1371/journal.pone.0174300 PMID: 28334047

45. Eguchi T, Bains S, Lee M-C, Tan KS, Hristov B, Buitrago DH, et al. Impact of Increasing Age on Cause-Specific Mortality and Morbidity in Patients With Stage I Non-Small-Cell Lung Cancer: A Competing Risks Analysis. J Clin Oncol. American Society of Clinical Oncology; 2017; 35: 281–290. https://doi.org/10.1200/JCO.2016.69.0834 PMID: 28095268

46. Van Laar RK, Tsuboi M, Ohira T, Saji H, Miyajima K, Kajiwara N, et al. Genomic signatures for predicting survival and adjuvant chemotherapy benefit in patients with non-small-cell lung cancer. BMC Med Genomics. BioMed Central; 2012; 5: 30. https://doi.org/10.1186/1755-8794-5-30 PMID: 22748043

47. Chen D-T, Hsu Y-L, Fulpi WJ, Coppola D, Haura EB, Yeatman TJ, et al. Prognostic and predictive value of a malignancy-risk gene signature in early-stage non-small cell lung cancer. J Natl Cancer Inst. Oxford University Press; 2011; 103: 1859–70. https://doi.org/10.1093/jnci/djr420 PMID: 22157961

48. Wistuba II, Behrens C, Lombardi F, Wagner S, Fujimoto J, Rao MG, et al. Validation of a Proliferation-Based Expression Signature as Prognostic Marker in Early Stage Lung Adenocarcinoma. Clin Cancer Res. 2013; 19.

49. György B, Hatzis C, Santt T, Hofstatter E, Aktas B, Puszta L. Multi-gene prognostic tests in breast cancer: past, present, future. Breast Cancer Res. 2015; 17: 11. https://doi.org/10.1186/s13058-015-0514-2 PMID: 25848861

50. Lassen U, Mau-Sørensen M, Hald Buhl U, Madsen MW, Balslev E, Pluim D, et al. Abstract CT154: Phase I dose-escalating PoC study to evaluate the safety and tolerability of LiPlaCis (liposomal cisplatin formulation) in patients with advanced or refractory tumors. Cancer Res. American Association for Cancer Research; 2016; 76: CT154–CT154. https://doi.org/10.1158/1538-7445.AM2016-CT154

51. Lassen UN, Knudsen S, Hertel PB, Kumler I, Nielsen D, Ejlersen B, et al. Use of microRNA to identify stage IV breast cancer patients to be targeted with phospholipase A2 disrupted cisplatin carrying liposomes: An ongoing phase I trial. | 2014 ASCO Annual Meeting | Abstracts | Meeting Library. ASCO Annual Meeting. American Society of Clinical Oncology; 2014. Available: http://meetinglibrary.asco.org/content/131616-144