Integrating proteomics into precision oncology

Leonie W. Wahjudi | Stephan Bernhardt | Khalid Abnaof | Peter Horak | Simon Kreutzfeldt | Christoph Heining | Simone Borgoni | Corinna Becki | Daniela Berg | Daniela Richter | Barbara Hutter | Sebastian Uhrig | Katrin Pfütze | Jonas Leichsenring | Hanno Glimm | Benedikt Brors | Christof von Kalle | Albrecht Stenzinger | Ulrike Korf | Stefan Fröhling | Stefan Wiemann

1Division of Molecular Genome Analysis, German Cancer Research Center (DKFZ), Heidelberg, Germany
2Division of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Germany
3German Cancer Consortium (DKTK), Heidelberg, Germany
4Department of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Dresden, Dresden, Germany
5Translational Functional Cancer Genomics, National Center for Tumor Diseases (NCT) and German Cancer Research Center (DKFZ), Heidelberg, Germany
6German Cancer Consortium (DKTK), Dresden, Germany
7Center for Personalized Oncology, National Center for Tumor Diseases (NCT) Dresden and University Hospital Carl Gustav Carus Dresden at TU Dresden, Dresden, Germany
8Division of Biosciences, University Heidelberg, Heidelberg, Germany
9Division of Applied Bioinformatics, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT) Heidelberg, Heidelberg, Germany
10Institute of Pathology, University Heidelberg, Heidelberg, Germany

Correspondence
Stefan Wiemann, Division of Molecular Genome Analysis, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg.
Email: s.wiemann@dkfz.de
Stefan Fröhling, Division of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Im Neuenheimer Feld 460, 69120 Heidelberg.
Email: stefan.froehling@nct-heidelberg.de

Funding information
European Commission, Grant/Award Number: 642691; Federal Ministry of Education and Research (BMBF), Grant/Award Number: FKZ:031A429

Abstract
DNA sequencing and RNA sequencing are increasingly applied in precision oncology, where molecular tumor boards evaluate the actionability of genetic events in individual tumors to guide targeted treatment. To work toward an additional level of patient characterization, we assessed the abundance and activity of 27 proteins in 134 patients whose tumors had previously undergone whole-exome and RNA sequencing within the Molecularly Aided Stratification for Tumor Eradication Research (MASTER) program of National Center for Tumor Diseases, Heidelberg. Proteomic and phosphoproteomic targets were selected to reflect the most relevant therapeutic baskets in MASTER. Among six different therapeutic baskets, the proteomic data supported treatment recommendations that were based on DNA and RNA analyses in 10% to 57% and frequently suggested alternative treatment options. In several cases, protein activities explained the patients’ clinical course and provided...
potential explanations for treatment failure. Our study indicates that the integrative analysis of DNA, RNA and protein data may refine therapeutic stratification of individual patients and, thus, holds potential to increase the success rate of precision cancer therapy. Prospective validation studies are needed to advance the integration of proteomic analysis into precision oncology.

**KEYWORDS**
genomics, precision oncology, proteomics, therapeutic decision making

1 | **INTRODUCTION**

Cancer is considered to be a "disease of the genome."\(^1\) Initiatives such as The Cancer Genome Atlas and the International Cancer Genome Consortium have been fundamental for a better understanding of the genomic basis of many frequent tumor entities.\(^2,3\) Together with numerous studies focusing on rare tumor entities,\(^4,5\) they have identified driver gene alterations.\(^6-8\) However, while some tumor entities are initially caused by recurrent alterations affecting specific pathways or even particular genes,\(^4,5,9,10\) the diversity of sequence variants, intratumor heterogeneity and cellular plasticity steadily increase with disease progression.\(^11\) These secondary events may be attributed also to therapies and contribute to increasing tumor aggressiveness.\(^12\) In precision oncology programs, such findings can now be exploited toward stratifying patients for therapy with targeted agents.\(^13,14\) Unbiased whole-exome sequencing (WES) and whole-genome sequencing (WGS) are thus increasingly applied in precision oncology programs, and are often complemented by transcriptome profiling.\(^15\)

The Molecularly Aided Stratification for Tumor Eradication Research (MASTER) program was launched at the National Center for Tumor Diseases (NCT) in Heidelberg, Germany, in 2013.\(^16\) This clinical precision oncology program enrolls young adults with advanced-stage cancers as well as patients with rare malignancies regardless of age for which standard therapeutic options are either exhausted or do not exist. Based on WES/WGS of tumor and matched normal tissue as well as RNA sequencing, potentially actionable lesions (eg, single nucleotide variants [SNV], copy number alterations [CNV], gene fusions, and aberrant transcription) and other informative alterations, for example, mutational load and signatures, are identified. A dedicated interdisciplinary molecular tumor board evaluates these findings and communicates individual treatment recommendations to the treating oncologist.\(^16,17\)

Despite the clinical impact MASTER and similar genome-driven precision oncology programs make, the lack of information on the biological relevance of genomic variants in the specific clinical context of an individual patient poses a major challenge.\(^1,18\) Genetic and transcriptomic data cannot predict protein levels and, even less, pathway activities in a way that would fully explain tumor biology.\(^19\) As a consequence, response to targeted treatment is often hard to predict, particularly for an individual patient who presents with a complex personal spectrum of genomic variants.\(^14\) Given that most targeted therapeutics act on proteins\(^20\) and that posttranslational modifications such as protein phosphorylation play crucial roles in cell signaling,\(^21\) it seems consistent to directly integrate proteomic and phosphoproteomic information into tumor profiling programs.\(^22,23\) Yet, broad proteomic analysis is only emerging to be considered in precision oncology.\(^24-26\)

Here, we have quantified the levels of 27 total and phosphorylated proteins in 134 tumor samples from the MASTER program in a retrospective proof-of-concept study. The cohort included more than 15 disease entities to represent a broad range of expression levels as well as phosphorylation states for the analyzed proteins. Protein targets were selected to reflect pathway activities in six interventional categories applied in the program. Depending on interventional category, proteomic data supported prior therapeutic proposals based on DNA/RNA analysis in 10 to 57 percent and was suggestive of alternative treatment options in most other cases. The findings suggest that proteomic information could help refine therapeutic decisions for advanced-stage cancer patients.

**What's new?**

Molecular tumor boards increasingly use DNA and RNA sequence information to identify genetic events in tumors to guide treatment for individual patients. Here, tumor samples from patients with advanced or rare cancers enrolled in the NCT MASTER precision oncology program in Heidelberg, Germany, were retrospectively assessed for expression and phosphorylation of proteins relevant to signaling pathways in six interventional categories applied in the program. Depending on interventional category, proteomic data supported prior therapeutic proposals based on DNA/RNA analysis in 10 to 57 percent and was suggestive of alternative treatment options in most other cases. The findings suggest that proteomic information could help refine therapeutic decisions for advanced-stage cancer patients.
2 | MATERIALS AND METHODS

2.1 | Clinical specimens

Tumor samples from 134 patients suffering from >15 distinct tumor entities (Table 1, Supplementary Table 1) who had been recruited into the MASTER precision oncology program for molecular characterization of tumor tissues (https://www.nct-heidelberg.de/forschung/nct-master.html) were analyzed in this study. All specimens were derived from fresh-frozen biopsies and underwent a standardized workflow for extraction of DNA, RNA and protein, all performed in a dedicated sample processing laboratory at the German Cancer Research Center (DKFZ) and NCT. For 118 of 134 (88%) patients, the same biopsy was performed for extraction of all analytes, while in 16 cases (12%), DNA, RNA and protein were derived from different biopsies, all taken from the same tumor and during the same visit of the patient in the clinic.

2.2 | Proteomic data

Expression levels of proteins and phosphoproteins were measured via reverse phase protein array (RPPA) technology and target-specific antibodies as previously described. Briefly, tissues were lysed in T-PER tissue protein extraction reagent (Thermo Fisher Scientific, Rockford, IL) containing Complete Mini Protease Inhibitor Cocktail (Roche Diagnostic, Mannheim, Germany) and PhosSTOP Phosphatase Inhibitor Cocktail (Roche Diagnostic, Mannheim, Germany) and adjusted to 2 μg/μL protein concentration. Lysates were spotted in technical triplicates on nitrocellulose-coated glass slides (Oncyte Avid, Grace-Biolabs, Bend, OR) using an Aushon 2470 contact printer (Aushon BioSystems, Billerica, MA). Five serial dilutions of selected tumor samples representing different tumor entities were spotted for testing of linearity of signals. Fast Green FCF (Merck, Darmstadt, Germany) intensities of spotted lysates were measured for every ninth slide to evaluate total protein levels and to rule out false measurements due to potential evaporation effects during the printing process. Target proteins were specifically detected using primary antibodies having been validated with western blotting prior to the study. All antibodies used throughout the study are described in Table 2. Arrays without primary antibodies were otherwise identically processed to serve as “blank” controls. Fluorescent signals were detected using an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE) at 700 nm and at 21 μm spatial and 16 bit optical resolution.

2.3 | Data analysis

Signal intensities were quantified using GenePixPro 7.0 (Molecular Devices, Sunnyvale, CA) and RPPA raw data preprocessing and quality control were performed using the RPPanalyzer R package. Fast Green FCF total protein intensities, “blank” controls and dilution series were used for data normalization and quality control. Only antibodies that showed significant enrichment over blank and had signals linear to total protein concentration from dilution series were included in the study.

Final protein expression levels were obtained by log transformation of signal intensities. These data are listed in Supplementary Table 2.

Signal intensities representing protein expression and phosphorylation levels were visualized in a heatmap using unsupervised hierarchical clustering with Euclidean distance and Ward’s agglomerative method. Pairwise protein correlations as well as correlations between proteomic and transcriptomic data were estimated using the Pearson correlation coefficient using GraphPad Prism 6.0. Pairwise protein correlations were plotted using the corplot R package using Tinn-R Editor 6.01.01.05 (https://nbcbib.uesc.br/tinnr/en/).

2.4 | Decision criteria for proteomic-based selection of interventional baskets

As RPPA generates relative protein expression data, signal intensities for every target (ie, total protein or phosphosite) were sorted for
TABLE 2  List of proteins and antibodies

| Primary antibodies utilized for target panel |  |
|---------------------------------------------|--|
| **Target protein** | **UniProt ID** | **Antibody** | **Specificity** | **Supplier** | **Antibody ID** | **Host** | **Basket RPPA** |
| AKT1 | P31749 | AKT_T308 | pT308 | CST | 4056 | Rabbit | PI3K-AKT-mTOR |
| AKT2 | P31751 | AKT_T308 | pT309 | CST | 4056 | Rabbit | PI3K-AKT-mTOR |
| AKT3 | Q9Y243 | AKT_T308 | pT305 | CST | 4056 | Rabbit | PI3K-AKT-mTOR |
| AKT1 | P31749 | AKT_S473 | pS473 | CST | 4058 | Rabbit | PI3K-AKT-mTOR |
| AKT2 | P31751 | AKT_S473 | pS474 | CST | 4058 | Rabbit | PI3K-AKT-mTOR |
| AKT3 | Q9Y243 | AKT_S473 | pS472 | CST | 4058 | Rabbit | PI3K-AKT-mTOR |
| ALK | Q9UM73 | ALK_Y1604 | pY1604 | CST | 3341 | Rabbit | Tyrosine kinases |
| ATM | Q13315 | ATM_S1981 | pS1981 | CST | 5883S | Rabbit | DNA damage response |
| BRAF | P15056 | BRAF | Total | CST | 9434 | Mouse | RAF-MEK-ERK |
| BTK | Q06187 | BTK | Total | CST | 3533 | Rabbit | Tyrosine kinases |
| CDK2 | P24941 | CDK2 | Total | Sigma-Aldrich | C5223 | Rabbit | Cell cycle regulation |
| CDK4 | P11802 | CDK4 | Total | CST | 2906 | Mouse | Cell cycle regulation |
| CDK6 | Q00534 | CDK6 | Total | SantaCruz | sc-177 | Rabbit | Cell cycle regulation |
| CHEK2 | O96017 | CHEK2 | Total | CST | 3440 | Mouse | DNA damage response |
| CTLA4 | P16410 | CTLA4 | Total | Abcam | ab134090 | Rabbit | Immune evasion |
| EGFR | P00533 | EGFR_Y1148 | pY1148 | CST | 4404 | Rabbit | Tyrosine kinases |
| ERBB2 | P04626 | ERBB2_Y1112 | pY1112 | Millipore | 04-294 | Mouse | Tyrosine kinases |
| ERBB2 | P04626 | ERBB2_Y1221_Y1222 | pY1221/pY1222 | CST | 2243 | Rabbit | Tyrosine kinases |
| ERBB3 | P21860 | ERBB3_Y1289 | pY1289 | CST | 4791 | Rabbit | Tyrosine kinases |
| ERBB4 | Q15303 | ERBB4_Y1162 | pY1162 | Epitomics | ab68478 | Rabbit | Tyrosine kinases |
| ERK1 | P27361 | ERK1_202_T202_Y204 | pT202/pY204 | CST | 4370 | Rabbit | RAF-MEK-ERK |
| ERK2 | P28482 | ERK1_202_T202_Y204 | pT185/pY187 | CST | 4370 | Rabbit | RAF-MEK-ERK |
| HRAS | P01112 | RAS_all | Total | Upstate | 05-516 | Mouse | RAF-MEK-ERK |
| KRAS | P01116 | RAS_all | Total | Upstate | 05-516 | Mouse | RAF-MEK-ERK |
| MET | P08581 | MET_Y1234_Y1235 | pY1234/pY1235 | CST | 3129 | Rabbit | Tyrosine kinases |
| NRAS | P01111 | RAS_all | Total | Upstate | 05-516 | Mouse | RAF-MEK-ERK |
| PDK1 | O15530 | PDK1_S241 | pS241 | CST | 3438 | Rabbit | PI3K-AKT-mTOR |
| PD-L1 | Q9NZQ7 | PDL1 | Total | Abcam | ab174838 | Rabbit | Immune evasion |
| PIK3CA | P42336 | PIK3CA_p85alpha | Total | Abcam | ab40755 | Rabbit | PI3K-AKT-mTOR |
| RAD51 | Q06609 | RAD51 | Total | CST | 8875S | Rabbit | DNA damage response |
| RB1 | P06400 | RB1_S780 | pS780 | CST | 3590 | Rabbit | Cell cycle regulation |

Primary antibodies utilized in validation

| **Target protein** | **UniProt ID** | **Antibody** | **Specificity** | **Supplier** | **Antibody ID** | **Host** | **Application** |
|--------------------|---------------|--------------|-----------------|--------------|-----------------|---------|----------------|
| EGFR | P00533 | EGFR | total | CST | 2646 | rabbit | Validation |
| EGFR | P00533 | EGFR_Y1068 | Y1068 | CST | 2234 | rabbit | Validation |
| EGFR | P00533 | EGFR_Y173 | Y173 | Abcam | ab32578 | rabbit | Validation |

Secondary antibodies utilized for detection of primary antibodies on RPPA

| **Target species** | **UniProt ID** | **Antibody** | **Fluorophore** | **Supplier** | **Antibody ID** | **Host** | **Application** |
|--------------------|---------------|--------------|-----------------|--------------|-----------------|---------|----------------|

(Continues)
TABLE 2 (Continued)

| Target protein | UniProt ID | Antibody | Specificity | Supplier | Antibody. ID | Host | Basket RPPA |
|----------------|------------|----------|-------------|----------|--------------|------|-------------|
| mouse          | n.a.       | F(ab’)2 fragment of IgG Alexa680 | Life Technologies | A21077   | goat         | Secondary antibody |
| rabbit         | n.a.       | F(ab’)2 fragment of IgG Alexa680 | Life Technologies | A21059   | goat         | Secondary antibody |

Note: pY, pS and pT: phosphorylated tyrosine (Y), serine (S) and threonine (T) residues, respectively. n.a.: not applicable. In column <Antibody>, amino acid positions are given according to annotation of the respective suppliers of antibodies, while in column <Specificity>, amino acid positions are given according to the respective UniProt entries. Suppliers: CST, Cell Signaling Technology; SantaCruz, Santa Cruz Biotechnology, Inc; Sigma-Aldrich, Merck Sigma-Aldrich; Upstate, Upstate Biotechnology, Inc; Fisher Scientific, Millipore, Merck Millipore; Epitomics, Abcam Epitomics.

3 | RESULTS

3.1 | Generation and initial validation of the proteomic dataset

Tumor samples were obtained from 134 patients recruited into the MASTER precision oncology program (https://www.nct-heidelberg.de/forschung/nct-master.html), reflecting a broad spectrum of cancer entities (Table 1, Supplementary Table 1). Twenty-seven of the 134 specimen were derived from metastases (20%) and 107 from primary tumors (80%). Twenty-seven proteins with central roles in six interventional baskets of MASTER (Tyrosine Kinases, PI3K-AKT-mTOR, RAF-MEK-ERK, Cell Cycle Regulation, DNA Damage Response, Immune Evasion) were analyzed for total levels (13 proteins) and phosphorylation (14 proteins/sites) (Table 2) in the tumor material. Expression levels of proteins and phosphoproteins were measured via reverse phase protein array (RPPA) technology. Signal intensities for the total and phosphoproteins having been measured are provided in Supplementary Table 2.

In a first-quality assessment of the data, the validity and reproducibility of our approach were ascertained. Initially, potential batch effects resulting from laboratory factors such as personnel, sample batches and processing dates could be excluded (data not shown). Next, we confirmed reproducibility of the applied method by testing several total proteins and posttranslational modifications in the same proteins using different specific antibodies and different replicate arrays. The correlation coefficient was low ($r = 0.2681$) when signal intensities of total and phospho-EGFR proteins were compared (Supplementary Figure 1A), reflecting the different activation states this protein may have. In contrast, pairwise comparisons of signals detected for three different activating phosphosites within the EGFR protein were consistently high ($r > 0.98$ in Supplementary Figure 1B-D). Data from quantification of two phosphosites in the AKT-proteins were similarly well correlated ($r = 0.9272$, Supplementary Figure 1E), even though positions T308 S473 are phosphorylated by PDK1 and mTORC1, respectively, and are associated with somewhat different functionalities.

The high-correlation coefficients observed with different antibodies and arrays ascertain high reproducibility of identifying distinct activating marks in the same proteins and, thus, of the RPPA.
technology. In contrast to EGFR, the correlation coefficient between phosphorylation sites in ERBB2 was weaker when the tested sites were indication of different functionalities (Supplementary Figure 1F). Although phosphorylation of residues Y1221 and Y1222 indicates activity of the ERBB2 receptor tyrosine kinase, residue phosphorylation at Y1112 has been associated with inhibition of phosphotransferase activity and marking of the protein for degradation.35 Catalytic activity of ERBB2 should precede inhibition of this phosphotransferase activity, which could explain the correlation coefficient of $r = 0.4881$ and that 6/6 patients above the 95th percentile and 14/28 above the 75th percentile for ERBB2_Y1112 were still recommended for the Tyrosine Kinas basket based on the proteomic data (Supplementary Table 3).

3.2 Correlation of signaling pathway activities within and between baskets

Next, we analyzed the pairwise correlation for all proteins having been tested in the respective interventional baskets. Consistent with the results obtained from the testing of different phosphosites within the same proteins, we observed strong correlations for most proteins and phosphoproteins within particular baskets (Figure 1). Correlation was also high between proteins of related baskets, for example, proteins in the baskets Tyrosine Kinas and PI3K-AKT-mTOR as well as RAF-MEK-ERK were strongly correlated ($P < .01$). Activated tyrosine kinase/PI3K/MAPK signaling was strongly correlated also with the expression levels of PD-L1 ($P < .01$) and this is in line with previous reports where PD-L1 was reported to be regulated by EGFR.39 However, CTLA4 and PDL1 protein expression did not correlate, which is consistent with the tumors and cell types expressing those proteins as well as with their different activities.40 Expression levels of neither protein correlated with tumor cell content (not shown).

The ERBB2_Y1112 and the ERBB4_Y1162 signals stood out in the Tyrosine Kinas basket as these two sites correlated best with RB1_S780 as well as with PDPK1_S241 ($P < .01$). These findings are in line with the functionalities that have been associated with the respective sites. ERBB2_Y1112 is connected with degradation of ERBB2 via recruitment of ubiquitin ligases35 while ERBB4_Y1162 has been related to induction of cell growth,41 which is in line with the observed correlation of the latter site with RB1_S780 ($P < .01$). PDPK1 phosphorylates AKT at residue T308 and is negatively regulated by 14-3-3 proteins.42 Phosphorylation of PDPK1 at S241 increases its interaction with 14-3-3 proteins and could thus also explain the poor correlation between PDPK1_S241 and ATK_T308 in the proteomic dataset (Figure 1).

3.3 Entity-independent distribution of protein-based therapeutic recommendations

We then performed unsupervised hierarchical cluster analysis, which grouped patients into four major clusters based on the signal intensities of proteins and phosphoproteins (Figure 2). Tumors in Cluster 1 had consistently low-signal intensities for almost all tested proteins. Since low expression of the proteins and phosphoproteins having been tested in the interventional basket DNA Damage Response has been suggested as indicator of potential actionability,36 this therapeutic basket was suggested particularly for patients in this cluster. At the other end of the spectrum, tumors in Cluster 4 showed high signals in almost all proteins. Consequently, a number of interventional options were suggested for these patients based on proteomic data. Tumors in Cluster 2 were characterized by strong signals particularly in

**FIGURE 1** Pearson correlation for the proteins/phosphoproteins analyzed. Proteins and modifications are grouped by respective interventional baskets these are associated with. The corplot R package34 was used to create a matrix that shows pairwise correlations ($P$ value of $< .01$). Blue color indicates positive correlations while negative correlations are in red color. Color intensity and the size of the bullets are proportional to the correlation coefficients.
proteins of the basket Cell Cycle Regulation, which is reflected in the proteomic-based recommendations (Figure 2). Tumors in Cluster 3 were low in Cell Cycle Regulation and RAF-MEK-ERK signaling, however, were high in Tyrosine Kinases, as well as PI3K-ATK-mTOR signaling. Also these observations are reflected in the proteomics-based therapeutic recommendations.

The levels of total protein and protein phosphorylation did neither cluster with cancer entity nor with the site the specimen had been taken from (ie, primary site or metastasis) or tumor cell content (Supplementary Figure 2). The lack of entity-based clustering is in line with previous observations showing that molecular driver events often occur across different tumor entities and corroborates the concept of the MASTER program that investigates therapeutic baskets independent of tumor entities. Yet, no interventional basket could be recommended for 28 patients based on proteomic data (Figure 2). From only two of these patients the tumor specimen had been derived from metastasized tumors while the other 26 had been biopsied from primary tumors and many (12/28) patients without recommendation clustered in a subcluster of patient-cluster 3.

3.4 Decision criteria for protein-based therapeutic recommendations

Although the heatmap shown in Figure 2 was suggestive of pathway activities for particular patients, a more objective approach was needed to guide proteomics-based treatment recommendations. Since RPPA generates relative quantitative information, we next ranked patients for each tested protein/phosphosite among all other patients in the cohort, as was previously suggested. We reasoned that proteins and phosphosites ranking in the upper and lower percentiles should have the highest predictive value for pathway activities. Accordingly, all tumors showing signals above the 95th as well as the 75th percentiles for each target were marked (solid blue and red lines, respectively, in Figure 3A, colored boxes for individual patients in Figure 3B, and numerically for all patients in Supplementary Figure 3). Based on this grouping, we defined decision criteria for protein-guided treatment recommendations: (a) signals and associated interventional baskets above the 95th percentile were given higher priority than signals mapping above the 75th percentile. (b) Active protein forms (ie, phosphorylated proteins) were given higher priority than total protein levels within a particular basket. (c) Concordant signals for different proteins within the same basket were regarded as strong indication of pathway activity. Based on this rationale and other criteria that were specific for particular baskets and are described in detail in Section 2, all patients were given scores that were finally used to recommend one or the other interventional basket for treatment of respective patients. A detailed listing of patients, scores and baskets is provided in Supplementary Table 3 and the recommended baskets are also indicated for individual patients in Figure 2.

FIGURE 2 Unsupervised hierarchical clustering of all tested proteins across all patients. The heatmap represents levels of total protein expression or phosphorylation levels of 134 tumor specimens. The z scores of log2-transformed expression levels of proteins and phosphoproteins (listed on the right) are color coded on a low-to-high scale (green-black-red) for individual patients (n = 134). Annotation bars inform on clinical data (cancer entities with coloring scheme, and tumor site with primary tumor vs metastasis). Treatment recommendations (Rec.) based on proteomic data vs data from DNA and RNA sequencing are annotated (recommendation: yes/no). Figure captions explain the respective color coding for expression levels, cancer entities, and recommendations.
received just a score of one in this basket by adding patients once they had a positive score also in either or both of the downstream targets phospho-ATK and phospho-ERK1/2. This way, 6 patients were additionally included (Supplementary Table 3).

Although the phosphorylation states of some tyrosine kinases are indication of the activity of the respective signaling pathways and, thus, also suggestive of a potential therapeutic relevance, proteomic criteria may not be of similar relevance for other baskets. The smallest number of patients was assigned to the Immune Evasion basket based on proteomic information (20 patients). Firstly, just two proteins were evaluated for this basket and these two proteins did not correlate well (Figure 1). This observation is in line with the literature having suggested that the predictive value of PD-L1 expression for successful immunotherapy is high, while that of CTLA-4 appears to be of lower relevance. Secondly, particularly in this basket, genomic analysis provides important information as to the therapeutic potential of immunotherapy for respective patients (eg, numbers of SNVs and indels, mutations in relevant genes). The value of proteomic information is, therefore, strongly affected by the number and predictive impact the marker proteins have in a particular therapeutic basket and should always be assessed in conjunction with molecular data from genomic analysis.

### 3.5 Partial overlap of protein-based vs genome- and transcriptome-guided therapeutic recommendations

To evaluate this further, we next wanted to assess the overlap of recommendations based on our proteomic data as compared to the

![Figure 3](https://example.com/figure3.png)

**Figure 3** Evaluation of protein/phosphorylation states of proteins in individual patients relative to the whole cohort. A, Signal intensities for total protein (CDK6) and phosphorylation (ERK1/ERK2_T202_Y204) states were ranked across the 134 tumors in the cohort (index). Solid blue lines represent the 5th and 95th percentiles while solid red lines indicate the 25th and 75th percentiles. Rank positions of patients presented in (B) are indicated. See Supplementary Figure 3 for similar plots of all antibodies and targets. B, Ranking of all analyzed proteins and phosphoproteins for three selected cases. Proteomic data are color coded according to the percentile for a respective protein relative to the whole cohort. Proteins are grouped according to interventional baskets. See Table 2 for details on antibodies and Supplementary Tables 2 and 3 for values and ranking of signal intensities for all individual targets and patients.
genome- and transcriptome-based MASTER recommendations. To this end, clinical reevaluation of all DNA/RNA data was performed according to current criteria (Supplementary File 1). Integrative analysis revealed both overlap and substantial differences between proteome- and genome-/transcriptome-based treatment recommendations, as depicted in Figure 2 and also in Supplementary Table 3. Proteomic data supported the MASTER recommendation based on DNA and RNA analysis alone in 10% to 57% of cases, depending on the respective interventional basket (Table 3). It has been noted that some recommendations based on Genomic/Transcriptomic data had a basket termed “Other” (Table 3). In this, any alterations in other potentially therapy-relevant pathways (e.g., Hedgehog/GLI, Delta/Notch, Wnt) or mechanisms (energy metabolism, drug transporters, mi/siRNA processing, hTERT) were summed up. The diversity and rarity of these mechanisms, however, rendered them irrelevant for the proof of principle study we present here.

Differences could, in part, be explained by the poor correlation between RNA expression and total as well as phosphoprotein levels, which was mostly insignificant (Supplementary Figure 4). It should be noted that genomic information had suggested a number of patients into particular baskets who scored low in proteomic analysis for any of the proteins within that basket. For example, patient 156 was in the 25th percentile for all phosphoproteins in the Tyrosine Kinases basket except ERBB4_Y1162 (in the 50th percentile) (Supplementary Table 3). A focal amplification in chromosome 10, which included the 

| Intervventional basket | Recommendations | Genomic/ transcriptomic # | Proteomic # | Agreement # | % Overlap |
|------------------------|-----------------|---------------------------|-------------|------------|-----------|
| Cell cycle regulation  | 18              | 48                        | 6           | 13         |
| DNA damage response    | 13              | 32                        | 4           | 13         |
| Immune evasion         | 18              | 20                        | 2           | 10         |
| PI3K-AKT-mTOR          | 26              | 37                        | 8           | 22         |
| RAF-MEK-ERK            | 21              | 38                        | 5           | 13         |
| Tyrosine kinases       | 68              | 49                        | 28          | 57         |
| None                   | 32              | 28                        | 5           | 18         |
| Other                  | 21              | 0                         | 0           | nd         |

Note: Numbers (#) of therapy recommendations for respective interventional baskets based on genetic/transcriptomic data as well as on proteomic data. Numbers of patients where recommendations based on genomic and proteomic data agreed (Agreement #) and the percentages of proteomic-based recommendations matching for individual patients (% Overlap). Genomic/transcriptomic classification comprised an interventional basket Other, which was not covered in the present study (nd, calculation not done).

For a more fine-grained analysis and to fully leverage the integration of proteomic data into MASTER, we next exemplarily evaluated three patients who were suggested for the Cell Cycle Regulation basket based on proteomic data (Figure 2B, Table 4, Supplementary Table 3). Patient 139 had been diagnosed with a chondrosarcoma more than 7 years prior to inclusion into MASTER. Since WES of a metastasis had revealed an amplification of the CDK4 gene and the RB1 gene was found to be intact and expressed, as judged by RNA sequencing, the molecular tumor board recommended therapy with a CDK4/6 inhibitor. In addition, amplifications of EGFR and ERBB3 were found, rendering tyrosine kinase inhibitors targeting EGFR or ERBB3 potential therapeutic candidates. The proteomic data specifically supported the option of tyrosine kinase inhibition (Figure 3B, Table 4). Several activating phosphosites on tyrosine kinases (ALK, ERBB2, ERBB3) ranked high. Also, phosphorylation of AKT1/2 at serine 473/474, indicative of active downstream signaling, was strongly elevated. In contrast, proteomic analysis did not show high expression levels of any of the proteins considered for the cell cycle regulation basket. In the clinic, the patient had received treatment with trofosfamide,
| Patient ID | Cancer entity                      | Master recommendation | Proteomics recommendation |
|------------|------------------------------------|-----------------------|---------------------------|
| 139        | Chondrosarcoma                     | Interventional basket  | Interventional basket     |
| 144        | Malignant peripheral nerve-sheath tumor (MPNST) | Cell cycle regulation | Tyrosine kinases DNA damage response |
| 133        | Melanoma                           | RAF-MEK-ERK           | RAF-MEK-ERK               |

**Interventional basket**

- **Tyrosine kinases**
- **Cell cycle regulation**
- **RAF-MEK-ERK**
- **Cell cycle regulation**
- **RAF-MEK-ERK**
- **Immune evasion**
- **Tyrosine kinases**

**Recommended therapy**

- *Lapatinib, afatinib*
- *CDK4/6 inhibition*
- *ERK-inhibition or pan-RAF inhibition*
- *CDK4/6 inhibition*
- *BRAF-inhibition, MEK-inhibition*
- *Checkpoint inhibition*
- *Lapatinib*

**Rationale**

- *Strong expression EGFR, ERBB3*
- *Focal amplification CDK4*
- *KRAS G12V mutation*
- *Amplification and high-level expression CDK4 and CDK6*
- *BRAF V600E mutation*
- *Hypermutation*
- *ERBB4 P172F mutation*

| Patient ID | Cancer entity                      | Master recommendation | Proteomics recommendation |
|------------|------------------------------------|-----------------------|---------------------------|
| 139        |                                    |                       |                           |
| 144        |                                    |                       |                           |
| 133        |                                    |                       |                           |

**Mutational signatures from genome sequencing**

- *COSMIC AC3 (BRCAiness)*
- *COSMIC AC7 (UV-irradiation)*

**Proteomics**

- **Rationale**
- **Interventional basket**
- **Tyrosine kinases**
- **DNA damage response**
- **Cell cycle regulation**
- **Tyrosine kinases**
- **Immune evasion**
- **Cell cycle regulation**
- **DNA damage response**

| Patient ID | Cancer entity                      | Master recommendation | Proteomics recommendation |
|------------|------------------------------------|-----------------------|---------------------------|
| 139        |                                    |                       |                           |
| 144        |                                    |                       |                           |
| 133        |                                    |                       |                           |

**Interventional basket**

- **RAF-MEK-ERK**
- **PI3K-AKT-mTOR**

**Rationale**

- *High-level expression RAS, strong expression BRAF, strong phosphorylation ERK1/2*
- *High-level expression PIK3CA, strong phosphorylation of AKT, PDK1*

**Note:** Presence of COSMIC signatures was assessed in genome sequence data following Alexandrov et al. 50.
doxorubicin, gemcitabine and docetaxel, trabectedin, pazopanib, high-dose ifosfamide and dacarbazine over the course of 5 years, which brought about disease stabilization as best response. Upon further progression, the patient did not benefit from reexposure to trofosfamide combined with the selective CDK4/6 inhibitor palbociclib. Proteomic data would have supported treatment with a pan-tyrosine kinase inhibitor and, thus, could have provided useful information to prioritize treatment options. Furthermore, targeting DNA damage response pathways was suggested at the protein level, as the expression of CHK2 was found to be low. Since WGS had identified 35% of SNV in the tumor to be explained by the AC3 signature indicative of defects in DNA double strand break repair by homologous recombination, there might have been a rationale for treatment with a PARP inhibitor.

For patient 144, diagnosed with a malignant peripheral nerve sheath tumor, DNA/RNA data had suggested treatment with an ERK or pan-RAF inhibitor combination therapy due to an oncogenic KRAS mutation, with a second option being a cell cycle inhibitor because of amplification and high-level expression of the CDK4 and CDK6 genes. The proteomic data corroborated the strong activation of the RAS-MEK-ERK pathway as total RAS and BRAF proteins were highly expressed and ERK1/2 were highly activated. Also, the amplification and high RNA expression of CDK4 and CDK6 genes were supported by high protein levels of CDK2, CDK4 and CDK6 as well as phosphorylation of RB1. This patient received chemotherapy with doxorubicin and ifosfamide followed by trofosfamide, inducing a durable complete remission. Proteomic information would have suggested additional therapeutic options, for example, targeting receptor tyrosine kinases and PI3K-AKT-mTOR signaling with signals for several proteins and phosphoproteins ranking in the upper 5th or 25th percentiles (Figure 3B, Supplementary Table 3), and could thus have provided a rationale for a potential alternative therapy in case of recurrence.

Patient 133 presented with malignant melanoma, first diagnosed 12 years prior to inclusion into MASTER. Previous treatment included surgical resection of the primary tumor, radiotherapy of spine and brain metastases, and CTLA4-directed immunotherapy (ipilimumab). Proteomic data would have suggested targeting of the cell cycle (high-level expression of CDK2 and CDK6). This rationale was corroborated by WGS, which found the RB1 gene to be wildtype and identified a somatic stop-gain mutation (E27X) in CDKN2A. Yet, the patient was initially treated again with an immune checkpoint inhibitor without durable clinical benefit and then switched to monotherapy with a BRAF inhibitor, as suggested by the presence of a BRAF V600E mutation. A subsequent combination of BRAF and MEK inhibition induced a partial remission of the disease for 6 months. Furthermore, the tumor carried an activating mutation in ERBB4 (P172F) suggesting treatment in the Tyrosine Kinases basket, for example, with lapatinib. The proteomic data indeed indicated an upregulation of ERBB4 activity; however, this was not reflected by a similarly upregulated signaling in downstream targets, that is, phosphorylation of ERK1/2 or AKT (Figure 3, Table 4, Supplementary Table 3). Instead, the proteomic data—if available at that time—would potentially have directed the molecular tumor board to a treatment recommendation involving cell cycle inhibition, consistent with the strong correlation between ERBB4_Y1162 and the inhibiting mark PDK1_S241 (RAF-MEK-ERK basket) and the proteins CDK6 and RB1_780 of Cell Cycle Regulation (Figure 1).

It must be noted that success of current targeted therapies in the basket cell cycle regulation (eg, using the CDK4/6 inhibitor palbociclib) depends on the expression of wildtype RB1 protein. Although proteomics can quantify the level of total and of phosphorylated RB1, protein and phosphoprotein data cannot reflect all inactivating mutations in this or many other genes. Hence, the combined analysis of tumor genomes, transcriptomes and proteomes is superior to the analysis of just one or two of these molecular levels.

4 | DISCUSSION

Here, we have investigated the potential value of proteomic data in the therapeutic decision-making process within an ongoing genomics- and transcriptomics-guided precision oncology program. To this end, we retrospectively studied the abundance and phosphorylation of 27 proteins and phosphosites in a cohort of 134 tumor samples. All patients were suffering from advanced-stage tumors and had been extensively pretreated. Comprehensive molecular genomic and transcriptomic data had been generated for all patients and used to make therapeutic recommendations. For the current study, we selected tumors representing the wide spectrum of tumor entities within the MASTER program and hypothesized that these would cover also a broad range of protein expression and phosphorylation states. Indeed, our data revealed a high dynamic range of expression/phosphorylation levels. Based on relative quantitative protein data, we devised a method for proteomics-deduced treatment recommendation. These recommendations were then compared to recommendations having been made by the MTB based on DNA/RNA data (our primary objective). Depending on the respective therapeutic baskets, proteomic data was supported by 10% to 57% of the previously made recommendations based on DNA/RNA analysis alone.

Although this small overlap may be surprising at first sight, the poor correlation between proteomic and transcriptomic data (Supplementary Figure 4) strengthens our reasoning that mRNA levels are not always good predictors of protein levels, and even less of protein phosphorylation states. Quantification of proteins and protein activation states should thus better reflect tumor biology than that of the transcriptome. This is in line with previous reports. Interestingly, neither total protein levels nor signals detected with phosphosite-specific antibodies reflecting phosphorylation states clustered with clinical parameters, like tumor entity or sample site (primary tumor vs metastasis). Even tumor cell content did not seem to impact the results, as there was no correlation between cellularity and signal intensities. However, more research is needed to assess a potential effect of this parameter because only 35 tumor specimens (26%) had a tumor cell content <60% in our study (Table 2, Supplementary Figure 2). Special techniques such as laser capture microdissection might be helpful to follow this up in greater detail.
Furthermore, signals for proteins and phosphoproteins with known functional association were highly correlated in our data, again independent of tumor entity. Our findings thus suggest that, at least for heavily pretreated patients, the original tissue type of primary tumors is not a main factor governing the activity of tumor cell signaling. Along these lines, our data support the pan-entity approach typically applied in basket trials, where molecular alterations and driver mechanisms within an individual tumor are evaluated across a range of different tumor types. Hence, therapeutic options are potentially enlarged beyond the space of treatment recommendations for separate histological entities.

The majority of tumor samples tested showed patterns of protein expression and/or phosphorylation that enabled therapeutic recommendations in most cases (106/134, 79%). Still, no treatment recommendations could be made for \( n = 28 \) (21%) patients based on proteomic data. This number was similar \( (n = 32) \) from molecular genomic analysis; however, neither proteomic nor genomic data could derive a treatment recommendation just for four patients. Only few tumors were consistently above the 95th or in the 5th percentiles, respectively, for the vast majority of proteins investigated. Although the former might benefit from drug combinations or broad-spectrum therapeutics (eg, patient 144 as described earlier), assignment to interventional baskets based on protein data should prove difficult for the latter and might be attributed in part to the rather focused set of proteins analyzed in this study.

As already implemented for genetic and transcriptomic data in MASTER, therapeutic recommendations should be prioritized according to the level of evidence underlying each treatment rationale, thus providing a comprehensive framework for clinical decision-making.\(^{16,17}\) Knowledge databases such as OncoKB\(^ {54}\) and CIVIC\(^ {55}\) are aimed to tackle this issue and are valuable resources for clinicians as they gather information about associations between specific mutations and clinical data. To bridge the gap between nucleic acids and proteins, open-access bioinformatics resources on proteomics, such as The Cancer Proteome Atlas (TCPA),\(^ {56}\) have recently been developed. However, all these datasets deal with probabilities and cannot inform on particular pathway activities within an individual tumor and patient.

Along these lines, our findings suggest that the combination of molecular testing of DNA, RNA and protein should further improve rational therapy recommendations made by molecular tumor boards (our secondary objective). This is exemplified by two of the three cases described in some detail earlier. While for patient 139, the DNA damage response basket had some indication with support from both protein and DNA data, this was not the case for patient 133 (Table 4). While DNA provides highly valuable information on single nucleotide as well as structural variants, RNA and, even more so, protein data inform on the presence and activities of a tumor’s functional modules.

Our study indicates that molecular evidence for therapeutic recommendations in advanced-stage cancer patients, as it is currently used in precision oncology, should be complemented by the inclusion of proteomic data. Amplification and increased transcription of genes like CDK4/6 or loss of genetic material, for example, of CDKN2A/B, do not always translate into activation of a particular oncogenic pathway. Levels of RNA and protein do not correlate well, and only direct protein analysis informs on expression levels as well as on activity states (eg, phosphorylation), suggesting this to be a superior readout of tumor physiology. This should require quantifying total as well as phosphorylated proteins to reflect protein and pathway activities. Proteomic data should thus help distinguish putative from true drivers and should be particularly valuable in cases where no strong driver events are detected by genetic analysis alone. To this end, other targeted as well as unbiased proteomic methods should be further examined in prospective trials for their value in clinical decision-making.\(^ {26,57}\)

However, the results presented here warrant validation in prospective trials toward refining knowledge on functional tumor states and, in consequence, improving treatment recommendations and the success of targeted treatment. Especially in cases where proteomic data contradicts genetic findings, more research is warranted to assess the respective value of both methods. To our knowledge, this is the first study that has generated proteomic data for patients who had entered an ongoing precision oncology trial, aiming to assess the potential additional value of proteomic data. Summing up, our study suggests that proteomic data, once positively evaluated in prospective trials in combination with genomic and transcriptomic data, might add valuable information to the decision-making process in interdisciplinary molecular tumor boards and should potentially be integrated into precision oncology programs.

ACKNOWLEDGEMENTS

We thank Daniela Heiss for her support in antibody validation. The authors thank the DKFZ-HIPO Sample Processing Laboratory, the DKFZ Genomics and Proteomics Core Facility, and the DKFZ-HIPO Data Management Group for technical support. We also thank K. Beck, K. Willmund, R. Eils and P. Lichter for infrastructure and development of MASTER within DKFZ-HIPO. Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

AS is member of the Science Advisory Board/Speaker’s Bureau of Astra Zeneca, Bayer, BMS, Eli Lilly, Illumina, Janssen, MSD, Novartis, Pfizer, Roche, Seattle Genetics, Takeda, ThermoFisher and is supported with grants from Bayer, BMS, Chugai.

ETHICS STATEMENT

Patient tissue samples were collected with informed consent under protocol NCT MASTER, S-206/2011 in accordance with its regulations and after approval by the Ethics Committee of Heidelberg University.

DATA AVAILABILITY STATEMENT

The proteomic data that have been collected in the course of this study is made available in Supplementary Table 2. Processed data for each patient and every basket, including recommendations based on genomic/ transcriptomic analysis, is presented in Supplementary Table 3. Other data that support the findings of this study are available from the corresponding authors upon request.
REFERENCES

1. Hyman DM, Taylor BS, Baselga J. Implementing genome-driven oncology. Cell. 2017;168:584-599.
2. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502:333-339.
3. International Cancer Genome Consortium, Hudson TJ, Anderson W, et al. International network of cancer genome projects. Nature. 2010;464:993-998.
4. Haller F, Bieg M, Will R, et al. Enhancer hijacking activates oncogenic transcription factor NR4A3 in acinic cell carcinomas of the salivary glands. Nat Commun. 2019;10:368.
5. Barthelmes S, Geddert H, Bolte C, et al. Solitary fibrous tumors/hemangiopericytomas with different variants of the NAB2-STAT6 gene fusion are characterized by specific histomorphology and distinct clinicopathological features. Am J Pathol. 2014;184:1209-1218.
6. Chang MT, Asthana S, Gao SP, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat Biotechnol. 2016;34:155-163.
7. Garraway LA, Lander ES. Lessons from the cancer genome. Cell. 2013;153:17-37.
8. Shumilov E, Flach J, Pabst T, et al. Genetic alterations crossing the borders of distinct hematopoietic lineages and solid tumors: diagnostic challenges in the era of high-throughput sequencing in hematopo-oncology. Crit Rev Oncol Hematol. 2018;126:64-79.
9. de Klein A, van Kessel AG, Grosveld G, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300:765-767.
10. Jones DT, Hutter B, Jager N, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. Nat Genet. 2013;45:927-932.
11. Gupta PB, P nastushenko I, Skibinski A, Blanpain C, Kuperwasser C. Phenotypic plasticity: driver of cancer initiation, progression, and therapy resistance. Cell Stem Cell. 2019;24:65-78.
12. de Klein A, van Kessel AG, Grosveld G, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300:765-767.
13. Jones DT, Hutter B, Jager N, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. Nat Genet. 2013;45:927-932.
14. Hyman DM, Piha-Paul SA, Won H, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature. 2018;554:189-194.
15. Horak P, Frohling S, Glimm H. Integrating next-generation sequencing into clinical oncology: strategies, promises and pitfalls. ESMO Open. 2016;1:e000094.
16. Horak P, Klink B, Heining C, et al. Precision oncology based on omics data: the NCT Heidelberg experience. Int J Cancer. 2017;141:877-886.
17. Leichsenring J, Horak P, Kreutzfeldt S, et al. Variant classification in precision oncology. Int J Cancer. 2019;145:2996-3010.
18. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature. 2012;483:100-103.
19. Akbani R, Ng PKS, Werner HMJ, et al. A pan-cancer proteomic perspective on the cancer genome atlas. Nat Commun. 2014;5:3887.
20. Pierobon M, Wulfkuhle J, Liotta LA, Petricoin E. Application of molecular technologies for phosphoproteomic analysis of clinical samples. Oncogene. 2015;34:805-814.
21. Khoury GA, Baliban RC, Floudas CA. Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. Sci Rep. 2011;1:90.
45. Cheng J, Demeulemeester J, Wedge DC, et al. Pan-cancer analysis of homozygous deletions in primary tumours uncovers rare tumour suppressors. Nat Commun. 2017;8:1221.
46. Jameson GS, Petricoin EF, Sachdev J, et al. A pilot study utilizing multi-omic molecular profiling to find potential targets and select individualized treatments for patients with previously treated metastatic breast cancer. Breast Cancer Res Treat. 2014;147:579-588.
47. Pierobon M, Silvestri A, Spira A, et al. Pilot phase I/II personalized therapy trial for metastatic colorectal cancer: evaluating the feasibility of protein pathway activation mapping for stratifying patients to therapy with imatinib and panitumumab. J Proteome Res. 2014;13:2846-2855.
48. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563-567.
49. Hu P, Liu Q, Deng G, et al. The prognostic value of cytotoxic T-lymphocyte antigen 4 in cancers: a systematic review and meta-analysis. Sci Rep. 2017;7:42913.
50. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415-421.
51. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov. 2015;14:130-146.
52. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13:227-232.
53. Mueller C, deCarvalho AC, Mikkelsen T, et al. Glioblastoma cell enrichment is critical for analysis of phosphorylated drug targets and proteomic-genomic correlations. Cancer Res. 2014;74:818-828.
54. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology Knowledge Base. JCO Precis Oncol. 2017:1-16.
55. Griffith M, Spies NC, Krysiak K, et al. CIVIC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. Nature Genet. 2017;49:170-174.
56. Li J, Akbani R, Zhao W, et al. Explore, visualize, and analyze functional cancer proteomic data using the cancer proteome atlas. Cancer Res. 2017;77:e51-e54.
57. Satpathy S, Jaehnig EJ, Krug K, et al. Microscaled proteogenomic methods for precision oncology. Nat Commun. 2020;11:532.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Wahjudi LW, Bernhardt S, Abnaof K, et al. Integrating proteomics into precision oncology. Int. J. Cancer. 2021;148:1438-1451. https://doi.org/10.1002/ijc.33301