REVIEW

The Pros and Cons of Incorporating Transcriptomics in the Age of Precision Oncology

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Abstract

The treatment of cancer continues to evolve toward personalized therapies based on individual patient and tumor characteristics. Our successes and failures in adopting a precision-oncology approach have demonstrated the utmost importance in identifying the proper predictive biomarkers of response. Until recently, most biomarkers were identified using immunohistochemistry for protein expression or single-gene analysis to identify targetable alterations. With the rapid propagation of next-generation sequencing to evaluate tumor tissue and “liquid biopsies,” identification of genomic biomarkers is now standard, particularly in non-small cell lung cancer, for which there is now an extensive catalog of biomarker-directed therapies with more anticipated to come. Despite these great strides, it has also become apparent that using genomic biomarkers alone will be insufficient, as it has been consistently shown that at least one-half of patients who undergo tumor genomic profiling have no actionable alteration. This is perhaps to be expected given the remarkable breadth of nongenetic factors that contribute to tumor initiation and progression. Some have proposed that the next logical step is to use transcriptome profiling to define new biomarkers of response to targeted agents. Recently, results from the WINTHER trial were published, specifically investigating the use of transcriptomics to improve match rates over genomic next-generation sequencing alone. In this review, we discuss the complexities of precision-oncology efforts and appraise the available evidence supporting the incorporation of transcriptomic data into the precision-oncology framework in the historical context of the development of biomarkers for directing cancer therapy.

The most contentious ongoing debate regarding precision oncology revolves around whether the incremental benefits to our patients are worth the high costs of testing and targeted treatments (1,2). Skeptics point to evidence such as SHIVA, a randomized, controlled trial that demonstrated no progression-free survival (PFS) benefit for off-label targeted therapies assigned by genomic testing compared with physician's choice of cytotoxic chemotherapy (3). Proponents of the precision-oncology approach counter with fundamental concerns about the appropriateness of the prespecified matches of alterations with targeted therapies, which might result in an underestimate of any effect (4). In contrast to SHIVA, the prospective MOSCATO-01 trial was positive, though the trial design and selected primary endpoint were quite different (5). An optimistic reading of the data highlights the fact that 33%

of patients who received a genomic-selected therapy benefited from it (6). A more sobering interpretation is that this represents only 7% of all patients screened, indicative of the fact that approximately one-half of the patients screened did not have an actionable alteration by the algorithm that was used in the study (5). Attempting to address this problem, results from the WINTHER trial were recently published, suggesting that incorporation of transcriptomic data can potentially be used to increase match rates over current strategies using genomic next-generation sequencing (NGS) alone (7). Whether such a strategy should be widely adopted in the near future is sure to be controversial, with valid questions about the cost-efficiency of such an approach needing to be asked and answered. In this review, we will provide a brief historical overview of precision oncology and discuss its complexities

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before examining the available evidence to evaluate the current role of transcriptomics within the precision-oncology paradigm.

Where We Have Been

The precision-oncology approach began in earnest with the use of immunohistochemistry (IHC) to identify specific biomarkers of response to therapies. The most notable early use of this methodology was in breast cancer, for which staining for estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2) is still conducted to predict responses to hormonal blockade and antibodies against HER2, respectively (8). Though it is an older modality, IHC remains an important part of the precision toolkit, and more recent important applications include the IHC-based assays of mismatch repair proteins (9) and programmed death-ligand 1 (PD-L1) (10), both of which are now used to predict responses to immune-checkpoint inhibitors.

In situ hybridization (ISH) is used as an alternative or complement to IHC to assess copy number variants, such as in the case of HER2 in breast cancer (11), and identify gene rearrangements and fusion events. Fusion products identifiable by ISH are believed to be ideal targets for precision therapies, because they are generally unique to abnormal cells and tend to be true oncogenic drivers. The most well-known example of this is the BCR-ABL translocation in chronic myeloid leukemia (CML) (12). CML was once one of the most recalcitrant hematologic malignancies, carrying a universally terrible prognosis (13), but following the development of tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL kinase, CML has now become one of the most treatable leukemias, with dramatic improvements in overall survival (14).

Where We Are

As technology has continued to advance, gene sequencing has led to the identification of further targets, and new drugs have been developed against these targets. For instance, BRAF V600E mutations have been targetable with BRAF and MEK inhibitors, with Food and Drug Administration-approved indications in melanoma (15), non-small cell lung cancer (NSCLC) (16), hairy cell leukemia (17), and anaplastic thyroid cancer (18). Mutations in epidermal growth factor receptor (EGFR) render advanced NSCLC more susceptible to targeted agents compared with platinum-based chemotherapy (19). The rapid proliferation of genomic targets has coincided with the availability of NGS, which allows for high-throughput sequencing of multiple targets in parallel. With this platform, sequencing can be rapidly performed using a wide variety of inputs, depending on preference and indication. These can include whole-genome sequencing, whole-exome sequencing, and targeted hotspots. Most frequently, this is conducted on tumor tissue but is paired in some instances with normal tissue to distinguish between somatic and germline mutations. In addition to analyzing either fresh frozen (FF) or archival formalin-fixed paraffin-embedded tumor tissue itself, so-called “liquid biopsies” of circulating tumor cells and cell-free DNA can be sequenced. NGS has been particularly useful in advanced NSCLC, where genomically selected therapies are available up front for EGFR mutations (19), BRAF V600E mutations (20), ALK fusions (21), ROS1 fusions (22), and NTRK fusions (23). Promising early-phase clinical trial data with selective TKIs against RET fusions and mutations (24) and MET exon

14 skipping mutations (25) suggest that these are also targetable in NSCLC and other tumor types. As more druggable mutations have been identified and more targeted agents are approved, NGS has become increasingly popular, especially with prior data suggesting that selecting therapies using more basic molecular profiling might benefit patients with otherwise refractory malignancies (26,27). This has culminated in the decision by the Centers for Medicare & Medicaid Services to cover FDA-approved NGS-based assays for those with advanced cancer (28). However, whether the benefits derived from genomic testing are justified by the costs continues to be debated (1).

The available evidence to date for broad-based genomic profiling and selection of treatment based on alterations found has not yielded impressive results. However, there are a variety of reasons for this that need to be understood and carefully considered rather than used as grounds to reject the idea outright. Aside from a handful of common mutations in TP53, PIK3CA, and KRAS that have eluded precision approaches, most mutations exist in the “long tail” of potentially targetable but infrequent alterations (29). This often precludes the preferred gold-standard randomized, controlled trial design. Excluding specific mutations that are enriched in specific diseases, such as BRAF V600E in melanoma, a very large number of patients need to be screened to identify a low-frequency mutation. For instance, to accrue 100 patients with a mutation seen in 1% of tumors, 10,000 patients would have to be screened. In common malignancies like NSCLC, this might be possible. In rarer malignancies, the task becomes functionally impossible. Thus, the approach has often been to design tumor-agnostic basket trials to be able to screen and accrue enough patients to make a meaningful conclusion (30). However, a flaw in this type of design is that many different alterations of the same gene—truncations, missense mutations, fusions, and copy number variations—are often combined into the same group, which may obscure the results, because these variants are all likely to be of different predictive value. Going back to the example of EGFR-mutated NSCLC, exon 19 deletions, exon 20 insertions, and T790M missense mutations will all respond very differently depending on the specific TKI used. Additionally, co-occurring alterations that may influence response to targeted treatments are often not considered in the matching of therapy. Furthermore, copy number increases due to true amplification may not be equivalent to those due to polyploidy, and these may not be distinguishable depending on the assay used (31). Grouping alterations in this fashion presumably improves enrollment but may mask positive signals. To further complicate matters, the same mutation may yield widely disparate responses to the same agents in different cancers. For instance, although BRAF V600E mutations have been shown to be targetable by BRAF and MEK inhibitors in multiple different cancer types (32), colorectal cancer with such a mutation has been markedly less responsive than other tumors to this approach (33). This is likely due to fundamental differences between malignancies in the tumor microenvironment and cell signaling when exposed to these inhibitors (34,35). Cases like this raise concerns about whether the design of basket trials is appropriate, though the upside is the possibility of a tumor-agnostic approval such as that for pembrolizumab in microsatellite-unstable tumors (36).

Owing to the scarcity of eligible enrollees, these basket trials are generally designed without an independent control arm and instead rely on metrics such as the PFS ratio, which uses each patient's outcome on the immediately preceding therapy as his or her own comparator (26). These types of measures are

imperfect in the sense that they are dependent on the substantial variability of prior treatments and responses (37). Because of ethical considerations, most precision-oncology trials enroll patients who have exhausted standard of care and are therefore heavily pretreated, meaning that the likelihood of seeing a signal is very slim, because with increasing lines of therapy, each successive treatment is likely to be of less benefit (38). However, a positive PFS ratio is frequently defined as a 30% improvement over the prior line of therapy, even though simply matching the prior response might be considered an accomplishment (37). Typical eligibility criteria for these trials also selects out malignancies that are either quick to progress or have a large number of approved options, as patients with either rarely reach the point of being appropriate for enrollment.

Another complexity of precision-oncology trials is that they are only as good as the targets and the drugs being tested. For instance, drugging a well-chosen target such as RET or NTRK with a highly selective and potent inhibitor has yielded exceptionally promising results (23,24). This is in stark contrast to generally unimpressive results trying to treat PIK3CA-mutated cancers with an mTOR inhibitor (4). Furthermore, targeted therapies may be more effective when used in combination rather than as monotherapies, particularly if there are multiple concurrent alterations (39,40), but most basket and umbrella trials are designed to test single agents. Finally, by appropriate design, these trials exclude patients with cancers that can be treated on-label, meaning that the most likely responders are omitted. All these mitigating factors are important to keep in mind when contemplating the rather modest results seen in many of these trials, such as the unsatisfying results from the tasisibis (41) and T-DM1 (42) arms of the NCI-MATCH trial.

Where We Are Going

As mentioned earlier, the MOSCATO-01 trial yielded encouraging results for the subset of patients that were able to be matched to therapy selected by genomic profiling, with 33% of these patients meeting the PFS ratio threshold of 1.3 (Table 1) (5). However, this represented only 7% of all patients screened for the trial, raising some important issues. First, only 199 (48%) of 411 patients with an identified actionable target received matched treatment on this study. This reinforces the notion that most patients who are rapidly declining or have too poor a performance status for treatment are unlikely to benefit from a precision-oncology approach, particularly if testing and interpretation takes too long. Inability to access the appropriate targeted agents, whether on study or off label, also nullifies the potential benefit of broad-based testing (43,44). Furthermore, only 411 (49%) of 843 patients who underwent successful molecular profiling of their tumors had an actionable target to begin with. Compared with other NGS-matched trials, this match rate is at the top end (Table 1), and more stringent matching criteria would drive this figure downward in exchange for better outcomes on treatment (3,45–47). Indeed, various algorithms to evaluate assigned treatment matches do seem to predict improved outcomes with higher scores (7,40). Regardless, match rates using genomic profiling will only improve with the identification of new targets and development of new drugs. However, outside of the development of effective treatments for the heretofore poorly druggable highest frequency alterations, the “long tail” distribution of mutations identified in cancer suggests that advances in this arena are likely to be marginal or confined to specific diseases (29). Thus, adding extra levels of

detail to the molecular portrait through other testing modalities will ultimately be required to boost match rates.

To address this last point, the WINTHER trial was conceived to evaluate the utility of transcriptomics as a supplement to genomic sequencing in advanced malignancies that have progressed following standard treatment (7). In this study, targeted NGS and transcriptomic profiling using dual-color microarrays of paired tumor and normal tissue were prospectively performed. Patients were either matched for treatment by NGS or by transcriptomic profile if no match was made by NGS. Of 303 patients who were consented for the study, 50 (17%) did not complete successful biopsies per protocol. A total of 107 (35%) were treated according to recommendations by their clinical management committee (CMC). Of these, 69 (23%) were matched to treatment by targeted genomic NGS and an additional 38 (13%) by transcriptomic data. Matches by RNA were determined using an algorithm that summed the concordance of the expression of each individual gene in the microarray to drug responses using the existing knowledge base.

No statistically significant differences between the DNA- and RNA-guided arms were observed in terms of disease control rate (23% vs 32%), defined in this trial as the sum of the rates of complete response, partial response, and stable disease lasting longer than 6 months. A PFS ratio of 1.5 was seen in 20% (14 of 69) of patients matched by NGS and 26% (10 of 38) matched by transcriptomics, which did not meet their prespecified primary endpoint of 50% and 40%, respectively (48). It is important to note that the threshold of 1.5 chosen in this study is much more ambitious than the norm of 1.3 (26), though post hoc analysis indicated that 25% of patients would have met the PFS ratio threshold of 1.3, which is only marginally higher compared with the 22% who met the threshold of 1.5. However, the authors point out that, of 17 additional patients who were not deemed evaluable by their study parameters because they were receiving treatment other than that recommended by the CMC, only 18% met PFS ratio thresholds of either 1.5 or 1.3.

Similar to other precision-oncology basket trials, the patients enrolled in this study were heavily pretreated, having undergone a median of three prior treatments, with 28 (26%) having been through five or more prior lines of therapy. Interestingly, being treated with two or fewer prior lines of therapy was independently predictive of improved PFS and OS, confirming that positive signals might be amplified by selecting patients who are not heavily pretreated. Most notably, the use of transcriptomic data increased the match rate by more than 50% in this study, from 23% to 36% in the treated patients. Though the NGS-based match rate is low compared with other studies (3,5,40,45–47), it is notable that 158 patients of the 303 enrolled (52%) had a CMC treatment recommendation, which is more reflective of the accurate match rate of their approach. Furthermore, if considering only the 253 patients who had successful biopsies both of tumor and normal tissue, the match rate increased to 62%.

However, considerable challenges associated with this type of approach remain. FF samples were required to improve the quality of the isolated RNA. Biopsies of normal as well as tumor tissue were needed for comparison, which is a major obstacle because the authors report that the most noteworthy factors leading to drop-out were inability to obtain a quality biopsy and patient withdrawal. Owing to the design of the study, some tantalizing questions remain unanswered, such as whether transcriptomic data might be combined with genomic data to improve prediction of responses.

Table 1. Tabulated results from notable tumor-agnostic basket trials

Trial	No. enrolled	No. successfully tested* (%)	No. with actionable alterations* (%)	No. treated with matched therapy* (%)	PFS2:PFS1 ratio >1.3† (%)	Objective response rate‡ (%)	Disease control rate‡ (%)
Standard genomics and IHC‡							
IMPACT (batch 1), Tsimberidou et al., 2012 (45)	1283	1144 (89)	460 (36)	211 (16)	NR	52 of 211 (25)	84 of 175 (48)§
SHIVA, Le Tourneau et al., 2015 (3)	741	496 (67)	293 (40)	99 (13)	NR	4 of 98 (4)	NR
PREDICT, Schwaederle et al., 2016 (47)	347	347 (100)	NR	87 (25)	NR	NR	30 of 87 (35)
IMPACT (batch 2), Tsimberidou et al., 2017 (46)	1436	1436 (100)	637 (44)	390 (27)	NR	43 of 381 (11)	111 of 381 (29)
MOSCATO-01, Massard et al., 2017 (5)	1035	843 (81)	411 (40)	199 (19)	63 of 193 (33)	22 of 194 (11)	NR
I-PREDICT, Sicklick et al., 2019 (40)	149	83 (56)¶	73 (49)	73 (49)¶	41 of 83 (49)¶	NR	21 of 69 (30)
Transcriptomics included#							
Von Hoff et al., 2010 (26)	106	86 (81)	84 (79)	66 (62)**	18 of 66 (27)	6 of 66 (10)	14 of 66 (21)
Radovich et al., 2016 (27)	168	168 (100)	NR	44 (26)	19 of 44 (43)	NR	NR
CoPPO, Tuxen et al., 2019 (44)	591	500 (85)	352 (60)	101 (17)	32 of 101 (32)	15 of 101 (15)	NR
WINTHER, Rodon et al., 2019 (7)	303	253 (83)	158 (52)	107 (35)	27 of 107 (25)	12 of 107 (11)	28 of 107 (26)

*Percentages relative to the number enrolled are listed in parentheses. Data not reported in the referenced publications are recorded as "NR." IHC: immunohistochemistry; PFS: Progression-free survival.

†Rates of the noted endpoints are calculated as the number meeting the endpoint divided by the number reported to be evaluable for that endpoint. Objective response rate is defined as the sum of complete and partial response rates. Disease control rate is the sum of the objective response rate and the rate of stable disease at the 6-month milestone in all studies, except in von Hoff et al., in which the rate of stable disease was assessed at 4 months.

‡Trials listed in the "Standard genomics and IHC" section used sequencing by genomic next-generation sequencing, copy number analysis using single-nucleotide polymorphism arrays or array comparative genomic hybridization, and IHC for marker detection.

§In IMPACT (batch 1), disease control rate excludes 36 evaluable patients with more than one target, for whom the stable disease rate at 6 months was not reported.

||In MOSCATO-01, the number enrolled excludes pediatric patients who were also consented for the study.

¶In I-PREDICT, the number successfully tested was not reported, so the number of treated patients was substituted. The number matched to therapy was not reported, so the number of evaluable patients treated with more than 10 days of therapy was used instead. The PFS ratio includes 10 patients treated with unmatched therapy, who were included as part of their matching score less than 50% group.

#Trials listed in the "Transcriptomics included" section included the above as well as transcriptomic analysis by either microarray or RNA sequencing.

**In von Hoff et al., matched therapies included conventional chemotherapy.

Soon after the results of the WINTHER trial were initially presented (48), results from the CoPPO trial combining genomic sequencing by whole-exome sequencing, copy number assessment by single-nucleotide polymorphism array, and transcriptomics by RNA sequencing (RNA-Seq) of FF samples to select treatments were published (44). Unlike in WINTHER, transcriptomic analysis of tumor samples was not compared with paired normal control individuals, and target positivity was instead defined by prespecified thresholds, with IHC confirmation of protein expression performed in most cases that proceeded to clinical trial based on RNA selection. Of the 500 patients who underwent biopsy after enrolling in the trial, 352 (70%) had a potentially actionable target identified. Of these patients, 145 (41%) had treatment recommended on the basis of genomic data, whereas that number rose to 207 (59%) if transcriptomic data were used. Notably, the match rate increase using transcriptomic data of approximately 50% is remarkably similar to that seen in the WINTHER trial. A total of 101 (29%) of the 352 patients with actionable targets were treated with matched therapy, and 100 (29%) received nonmatched therapy. A total of 32 (32%) of the 101 patients receiving matched therapy had a PFS ratio greater than 1.3, but this rate was not reported for the patients receiving nonmatched therapy. Partial responses were seen in 15 (15%) patients, with no complete responses.

Unfortunately, problems remain with this approach. Despite a more manageable biopsy protocol than WINTHER, 67 (13%) patients experienced biopsy failure, though 27 (5%) did undergo a successful rebiopsy. Though the objective response rate (ORR) of patients receiving matched therapy in CoPPO was low (15%), this was comparable with that seen in WINTHER (11%). If calculating the ORR as a percentage of the intention-to-treat

population, the ORR for both trials was dismal, at 3% and 4% for CoPPO and WINTHER, respectively. However, both trials reported inability to procure drugs on study or off label as substantial barriers to accurately assessing the benefit of this approach (43,44). In CoPPO, only 29% of patients received matched treatment compared with 68% in WINTHER. Furthermore, ORR may not be an appropriate measure of benefit in the typical patient population enrolling in these types of trials because it does not account for the diversity of malignancies being assessed or the heavily pretreated nature of the majority of these patients.

Discussion

Though the use of transcriptomic data to match patients to targeted agents in tumor-agnostic precision-oncology basket trials is relatively new, mRNA assays have already been used for some time to guide treatment in cancer. Panel-based gene expression profiling (GEP) has been used for molecular subtyping and risk stratification of various cancers for many years. The most successful example is the use of gene signatures to further delineate node-negative ER-positive breast cancers into low- and high-risk subgroups (49). Based on this observation, a 21-gene panel has been used to define a subset of patients with ER-positive breast cancer who are likely to benefit from the addition of chemotherapy to hormonal blockade (50). Although molecular subtyping by GEP has been performed in other cancers, the clinical benefit of this approach outside breast cancer is questionable, though studies are ongoing. In prostate cancer, it is unclear whether GEP adds any clinically significant prognostic information to IHC-based assays (51,52). Early results from

the COMPASS trial indicated that pairing whole-genome sequencing and RNA-Seq could generate a more complete molecular portrait of pancreatic ductal adenocarcinoma according to previously reported mRNA signatures. However, much simpler surrogate markers correlated with these subtypes as well, including GATA6 expression by ISH and tumor grade and stage (53). Although these are preliminary results, they do echo a report from prostate cancer, for which IHC of PTEN alone compared favorably with GEP encompassing more targets (51). In colon cancer, a prospective trial of GEP validated its ability to stratify patients by risk of recurrence independent of clinical and pathologic staging, but it was unable to predict benefit from the addition of oxaliplatin (54). These findings are in direct contrast to what has been shown in breast cancer, for which the prospective TAILORx trial was able to demonstrate that GEP can be used to predict not only who might benefit from the addition of chemotherapy but also who can safely be spared from it (55).

As demonstrated in WINTHER (7) and CoPPO (44), the addition of broad-based RNA assays does appear to have an incremental benefit over genomic data alone in assigning patients to matched therapy (Table 1). Their utility is likely to improve with the development of more sophisticated matching algorithms, particularly those that can account for complex gene expression signatures that indicate activation of targetable pathways, as opposed to evaluating single-gene expression. Furthermore, mRNA assays can be used to identify splice variants that may not be detectable by genomic NGS. Androgen receptor splice variant 7 (56) and MET exon 14 skipping mutations (25) have already been demonstrated to have clinical relevance, and more actionable splice variants are likely to be identified in the future. Finally, given that changes in gene expression without genomic alterations are important in determining the function of immune cells, immune profiling using RNA-based assays is being actively investigated and may succeed at predicting responses to immunotherapy where IHC and genomic NGS have failed (57). However, these should be considered theoretical benefits until more data are available, and the use of broad-based transcriptomic analyses to direct therapies at this time should be confined to investigational studies.

Many critical questions about how to generate and interpret transcriptomic data need to be answered before we can seriously consider their standard use. Is the appropriate comparator normal tissue from the same patient or historical data from the same type of tumor in other patients? Are prespecified expression thresholds more appropriate than relative expression compared with normal or historical control individuals? Can archival formalin-fixed paraffin-embedded samples be a reliable source of RNA for transcriptomics? How variable are transcriptional changes over time and when is the proper time to obtain a specimen for testing? Does unbiased RNA-Seq yield more predictive information than panel-based assays? Does treatment selection based on these biomarkers improve outcomes?

In the interim, there are many other opportunities for improvement using testing modalities that are currently widely available. Standardizing assays among multiple providers will be critical. Many different tests may be available for the same analyte, though only specific ones might be validated as a predictive biomarker. Examples include assays of microsatellite instability, tumor mutational burden, and PD-L1 expression. IHC for PD-L1 expression has been particularly troublesome; different antibodies and scoring systems have been validated for different drugs, diseases, and even treatment indications, with varying degrees of overlap (10). Discordant findings have been noted even with relatively routine IHC-based assays due to test-

to-test and lab-to-lab variability as well as sampling differences arising as a result of tumor heterogeneity (58). These issues are compounded when dealing with massively parallel high-throughput NGS-based assays.

Targeted NGS panels provided by different vendors do not have standardization of the genes or alterations tested. These subtle differences may lead to erroneous negative calls. Also, there is no standard variant allele frequency (VAF) cutoff for determining if a mutation is actionable, because the interpretation of VAF depends heavily on the tumor purity of the submitted sample and the specific assay. For instance, a VAF less than 1% might be considered irrelevant in tissue-based NGS of a 100% pure biopsy specimen but could be potentially meaningful if the tumor purity is below 10% or if dealing with cell-free DNA. Similar problems exist in the interpretation of copy number alterations and can be anticipated if considering transcriptomic data (31,42). Indeed, between WINTHER and CoPPO, two different standards were used: relative gene expression to normal as defined by dual-color comparative hybridization vs prespecified thresholds of gene expression.

Treatment recommendations included in most commercial NGS reports are only as good as the algorithm and evidence database that are used, and these vary depending on the vendor and can change without notice. Unfortunately, standardizing matching protocols is unlikely given the rapid evolution of available evidence, but this also severely limits cross-trial comparisons. Increasing access to multidisciplinary molecular tumor boards encompassing opinions from a wide range of clinicians and basic scientists, particularly physician scientists, is critical to more flexible and thoughtful interpretation of the vast amounts of data provided by genomic profiling (59). However, these are subject to institutional biases and personal opinions, which lead to more variability, though the incorporation of artificial intelligence might mitigate some of this. As NGS has become more common, there has also been an exponential increase in the number of variants of unknown significance, which confounds the interpretation of the data (60). To complicate matters, "liquid biopsies" may be falsely positive for known cancer-associated mutations as a result of clonal hematopoiesis of indeterminate potential (61). Furthermore, multiple concurrent alterations are frequently found, but most basket trials are designed to target only one alteration, often with monotherapies.

Recently, the I-PREDICT trial demonstrated that it is feasible and probably preferable to rationally design combinations of therapies when multiple different targets are identified, providing evidence for the idea that prior basket trials may underestimate the benefit of precision-oncology approaches (40). Further investigation to demonstrate the efficacy and tolerability of combination approaches will be critical, especially anticipating that future incorporation of nongenomic biomarkers are likely to increase the rate of finding multiple concurrent targets. However, before combinatorial approaches can be seriously considered, the problem of access to drugs recommended on study or off label remains a daunting one, with many trials reporting that less than one-half of the patients received the treatment they were matched to (Table 1) despite the fact most recommendations were monotherapies.

As discussed earlier, several different studies have demonstrated that simple surrogate biomarkers may exist that are functionally equivalent to more complex assays. A natural extension of this may be the use of demographic data or other components of clinical data as part of the treatment selection process. Racial disparities are already known to be strong

influences on disease behavior and responses to treatment (62). Further investigation of these influences may yield more immediate benefits than dealing with the myriad of complicated problems that arise when trying to make sense of large swaths of “-omics” data.

Perhaps the most important opportunity for improvement is for clinicians to be as up to date as possible on new targeted indications and drug approvals. A recent study of NGS used in the community setting to direct therapy for NSCLC concluded that NGS provided no statistically significant survival benefit over routine testing for EGFR and ALK alterations (63). However, only 36 (29%) of 125 patients who would have been eligible for matched therapy based on the current evidence, particularly based on mutations in targets other than EGFR and ALK, actually received it (64). In another study of NGS in advanced NSCLC, 81 (72%) of 113 patients were treated with genomic-matched therapy, with disease control observed in 36 (86%) of 42 evaluable patients (65). Though survival data are not yet available for this trial, it is clear that improving awareness of drug-target matches with the strongest backing evidence can dramatically improve patient outcomes. However, this is far easier said than done given the rapidity with which new drugs and indications are approved.

The WINTHER and CoPPO trials represent an important proof of principle that transcriptomic data can be generated from patient tumors and used to improve the match rate over genomic profiling alone. The methodology used in WINTHER comparing mRNA expression in FF biopsies of tumors against matched normal tissue from the same patient likely improves the quality of the data being used to assign matches. However, it may be unrealistic in standard practice to subject patients to such an arduous biopsy protocol, particularly if they are clinically deteriorating, as many of these patients are. The ability to increase the match rate using transcriptomic data is notable, especially because match rates using genomic NGS have capped out around 50%. However, the additional cost and difficulty in obtaining high-quality transcriptomic data at this time are not justified by the marginal benefit and this is not yet ready for widespread adoption. That said, this is an area that is worthy of continued investigation and is likely to yield better results over time.

Notes

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