Identifying Rare Events in Rare Diseases

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Running Title: Predicting the Future in Neuroblastoma

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Summary

Utilizing genomic signatures from diagnostic tumor samples to forecast clinical behavior and response to therapy has long been a goal, and we are now poised to further refine how we can identify the relatively rare patients with aggressive neuroblastoma masquerading as patients with a more benign form of the disease.
In this issue of *Clinical Cancer Research*, Oberthuer and colleagues report a new gene expression classifier that has the potential to reliably identify children currently classified as having low- or intermediate-risk neuroblastoma with a significantly increased risk of death (1). Conversely, the authors also demonstrate the classifier’s ability to reliably identify low- or intermediate-risk neuroblastoma that will be cured with current treatment guidelines of surgery alone or relatively minimal amounts of adjuvant chemotherapy. They propose that this classifier is ready to be validated in a prospective clinical trial.

Until cancer therapy is tailored to the individual patient, risk stratification will remain an important tool in clinical oncology, allowing groups of patients with better or worse outcomes to receive an appropriate treatment plan (as illustrated in Fig. 1). This is especially true for children with neuroblastoma where about 25% of children have disease that spontaneously regresses and/or differentiates, while about 50% have metastatic disease that is often refractory to even the most intensive treatment regimens (2). To this end, genomic markers have been used for over 30 years to stratify children with neuroblastoma as having either a low, intermediate, or high risk of relapse (and death). Currently used markers include *MYCN* amplification, DNA index, loss of chromosome arms 1p or 11q, and more recently, the presence or absence of whole chromosome numerical aberrations and segmental chromosomal aberrations (3-5). Indeed, MYCN amplification status provides the seminal example of the prognostic utility of a genomic biomarker and has been used to identify patients with aggressive neuroblastoma since the mid-1980s. While these markers provide a rigorous classification into low-, intermediate-, and high-risk groups when combined with clinical
features, there are still patients assigned to the non-high-risk groups that have tumors that ultimately behave in a high-risk fashion.

The authors developed a gene-expression-based classifier from a training set of 75 cases and evaluated its performance in 634 separate cases. Although the classifier performed well relative to the clinical risk groups across the entire cohort, its predictive power was most dramatic in the low- and intermediate-risk group of patients (perhaps due to an overrepresentation of these groups in the overall cohort). In these non-high-risk groups, the gene expression classifier correctly identified the majority of cases with 100% overall survival and the minority of cases with overall survivals as low as 51%. The classifier remained significant in multivariable models, and the authors ultimately propose therapy modifications to prospectively test their classifier in a clinical trial.

Prognostic signatures based on gene expression microarrays have been proposed for many human cancers over the last 10-15 years, including neuroblastoma (6-9), but there are many logistical issues influencing clinical applications and current FDA-approved assays are limited to breast cancer patients (10, 11). The barriers to implementing such an assay are sizable, but we are rapidly approaching a time where they could be overcome. First, the cost of the microarrays is now no longer prohibitive and, in many cases, is competitive with alternative testing modalities. Second, it still takes several weeks from after the tissue collection to obtain a result, but our experience with currently used genomic markers shows that trials can be developed to account for this inherent (and steadily decreasing) delay. Third, tissue collection in multi-center trials is critically important when RNA is to be preserved (as opposed to DNA-based assays), but this too can be done with proper planning and tissue collection.
standard operating procedures. An inevaluable rate of 5-10% (as estimated by the authors) should not significantly impede their planned prospective study. Finally, any microarray approach will be confounded by sampling error due to tumor heterogeneity. The authors propose performing two expression profiles for each tumor in an attempt to mitigate this error. This will double the cost of the assay and somewhat reduce the possibility of misclassification. Deep sequencing approaches may ultimately be able to dissect this heterogeneity, but all of the previously discussed barriers would come back into play.

The classifier proposed by Oberthuer and colleagues (1) has the potential to improve outcomes and adverse events for children with low- and intermediate-risk neuroblastoma, and it is clearly appropriate to test this classifier in the prospective trial proposed by the authors. It would have been interesting to understand the relative merit of the gene expression classifier compared to DNA-based measures of mutation and structural variation. It is clear that structural chromosomal rearrangements also have prognostic power in the non-high-risk group of patients (5), and perhaps DNA- and RNA-based genomic classifiers are complementary.

While impacting outcome for children with non-high-risk neuroblastoma is clearly an important goal, it is also clear that the major unmet need is to improve outcomes for children with high-risk disease. These patients continue to have poor clinical outcomes despite a several-fold increase in treatment intensity over the last two decades. Children receive highly dose-intensive therapy, and there are no reliable predictors of therapeutic response or survival within the high-risk group of patients. Approximately 10-15% of high-risk patients show significant de novo resistance to current chemotherapy, but we
currently have no mechanism to identify these patients or alter therapies until it is perhaps too late. This study takes a step towards discovering such a classifier, but clearly more collaborative work will be required to define robust genomic biomarkers of high-risk neuroblastoma outcomes that can be integrated into current highly complex treatment regimens. Furthermore, once one can predict which patients have inferior outcomes, one has to have an evidenced-based way to respond to the information. The fact that activating mutations in the ALK tyrosine kinase protein are associated with more aggressive disease (12), and can be specifically targeted therapeutically (13), offers at least one example of a rational therapeutic intervention based on the identification of a prognostically unfavorable genomic biomarker. Thus, an integrated approach that utilizes the most prognostically relevant genomic data, perhaps as measured next generation sequencing technologies, may provide the most robust classifier for all patients with neuroblastoma.

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**References**


Figure 1. Oberthuer and colleagues (1) present evidence that gene expression signature can identify rare patients with aggressive neuroblastoma (red) masquerading as patients with benign disease based on currently used clinical and biological features (top). By using gene expression signatures (middle), the investigators propose to prospectively segregate low- and intermediate-risk neuroblastoma patients into a large population curable with minimal, if any, therapeutic intervention (bottom left) and a smaller population enriched with patients having a more aggressive form of the disease who may benefit from cytotoxic therapy (bottom right).
Figure 1:

Patients with low- or intermediate-risk neuroblastoma

Gene expression signatures

Large population curable with minimal, if any, therapeutic intervention

Smaller population, including patients who have a more aggressive form of the disease, that may benefit from cytotoxic therapy
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