Cangelosi et al sought to evaluate the prognostic value of the level of Nucleolin (NCL) mRNA expression in neuroblastoma, and its potential clinical utility in risk stratification. They provided evidence that NCL is an independent (adjusting for age, INSS stage, MYCN status) novel prognostic biomarker. This research is significant because prognostic biomarkers are used to assign patients with neuroblastoma to the appropriate level of therapeutic intensity. In cooperative groups like Children’s Oncology Group and International Society for Pediatric Oncology-Neuroblastoma, and throughout the world, treating physicians follow validated risk stratification (‘classifiers’) based on biomarkers that are prognostic of survival, to assign patients to low-, intermediate-, and high-risk groups. Ideally, classifiers are statistically developed based on evaluation of all possible biomarkers in the largest possible patient cohort.2–5 In general, patients in low-risk are assigned to surgery and observation, intermediate-risk to response-based chemotherapy (usually 2–6 cycles), and high-risk to intensive multi-modality including autologous stem cell transplant and immunotherapy with anti-GD2 antibody. The International Neuroblastoma Risk Groups (INRG) Task Force developed an internationally accepted pre-treatment risk stratification; biomarker and outcome data for >24,000 patients are stored in the INRG Data Commons (https://commons.cri.uchicago.edu/inrg).

The ongoing process of identifying prognostic biomarkers in neuroblastoma began decades ago with age, stage, and MYCN status.6–8 Despite identification of new biomarkers, age, stage, and MYCN status remain the most highly prognostic and are the foundation of current neuroblastoma risk stratification.

Cangelosi et al have provided evidence that NCL is an independent novel prognostic biomarker for neuroblastoma, whereby high mRNA expression of NCL was associated with lower overall (OS) and event-free survival (EFS). NCL remains statistically significant even after including age at diagnosis, INSS stage, and MYCN status in the Cox proportional hazards regression models (Table 1). The effect size (hazard ratio [HR]) of NCL on outcome, relative to age, stage, and MYCN status, is modest: HR = 1.17 vs HR of 2–3, respectively (Table 1). It is not uncommon for a neuroblastoma biomarker to have a strong prognostic effect in univariate analysis, but modest when compared to age, stage, and MYCN status in multivariable analysis. Further analyses will be required to see how the effect of NCL on survival compares to that of other neuroblastoma biomarkers.

The analyses of Cangelosi et al included a discovery cohort (n = 20) and a validation cohort (n = 786). Alternative approaches could have been used by Cangelosi et al to strengthen the validity of the NCL cut-off they identified: 1) randomly partition the n = 786 into separate test and validation cohorts; and 2) utilize the Kaplan–Meier scan method, which maximizes the difference between the OS curves, instead of the elbow method, which maximizes the difference in NCL expression level between the two subgroups. The OS difference is relevant to prognostic stratification; the expression level difference is not.

Cangelosi et al assert that NCL should be used clinically in risk stratification. Before this can be considered, a) a consensus platform/approach will need to be selected (and CLIA certified if used in the USA); b) the expression cut-off value of 0.838 should be cross-validated across multiple labs to ensure it is clinically meaningful and reproducible; and c) its prognostic strength should be validated in a larger cohorts, compared head-to-head with other NB prognostic biomarkers, including serum lactate dehydrogenase (LDH), serum ferritin, segmental chromosomal aberrations (11q LOH, 1p LOH), ALK, ploidy, and histologic factors mitosis-karyorrhexis index (MKI), tumor grade of differentiation, and diagnostic category. Ideally, the latter could be accomplished in the INRG Data Commons; it is understandable that Cangelosi et al did not have the data on other biomarkers to perform those analyses.

Attempts to stratify the high-risk neuroblastoma population have been made, but did not test novel biomarkers.9–10 With funding from Solving Kids’ Cancer UK, the ongoing BORNEO (BioMarkers in High Risk NeurOblastoma) project is conducting a worldwide search for new prognostic neuroblastoma biomarkers to add to INRG, performing head-to-head comparisons of their relative prognostic strength to stratify the high-risk neuroblastoma population.10 Cangelosi et al have...
compared the prognostic ability of NCL to age, INSS stage, and MYCN status, but comprehensive analyses comparing NCL to all the biomarkers in the INRG database will be needed. Cangelosi et al will be invited to share their data on the NCL biomarker for comparisons in the BORNEO project.

Cangelosi et al provide strong evidence for pursuing further research of NCL expression as a potential neuroblastoma clinical biomarker, though it remains to be seen whether the magnitude of the HR for NCL will be clinically meaningful and useful in risk stratification, and if the optimal cut-off is reproducible. It is possible there may be some subgroups of patients where NCL expression is as good, or better than, existing prognostic factors. Further research is also warranted to determine whether NCL is a predictive biomarker in addition to being prognostic, i.e., a potential target for anti-viral or anti-tumor strategies. Cangelosi et al emphasize that, “the identification of novel prognostic biomarkers and more active and less toxic therapies are urgently needed.” Discoveries such as the one by Cangelosi et al will be critically important to improved risk stratification and treatment outcome in neuroblastoma. Until such time as predictive biomarkers and drugs for targeted therapy are available for neuroblastoma, research on improved prognostic stratification should continue, as one means to improve neuroblastoma outcome and minimize toxicity.

Contributors
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