

INTERNATIONAL NEUROBLASTOMA RISK GROUP
TASK FORCE
PROJECT PROPOSAL

Thank you for your interest in INRG data.

Please send your completed proposal and any questions to scohn@peds.bsd.uchicago.edu

Proposal Title	International integrated analysis to identify markers of poor survival in high-risk neuroblastoma
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NOTE:

- Please limit your request to 5 pages
- If you would like to perform the analysis locally, in lieu of using an INRG statistician, please include the CV of your biostatistician and provide a detailed statistical plan.

Please format your project proposal as follows:

This proposal includes three steps: Systematic Review, INRGdb work and Integrated Cohort (see figure 1)

1. Specific Aims

Systematic Review Phase

- To identify all definitions/endpoints that have been utilized in attempting to define a poor risk subgroup within high risk neuroblastoma patients (so called “ultra-high risk” [UHR])
- To identify a set of “optimal” prognostic factors (biomarker-based or clinical factors) with the highest performance in the systematic review (e.g. best hazard ratio for the case of survival analyses)
- To conduct a meta-analysis of the prognostic factors (if data quality allows)

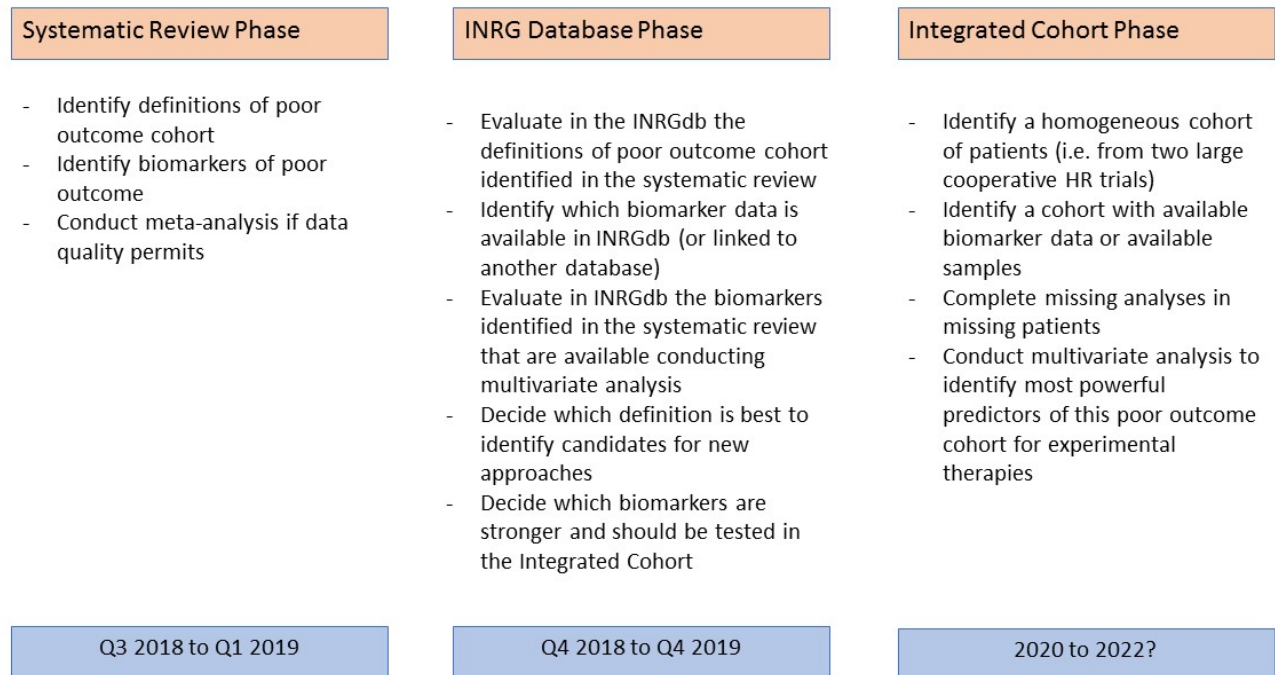
INRGdb Phase

- To evaluate in the INRGdb cohort of high risk neuroblastoma patients the definitions/endpoints of the poor outcome cohort identified in the systematic review.
- To identify and list which biomarker data is available in INRGdb (or linked to another database)
- To evaluate (by conducting multivariate analysis) in the INRGdb cohort the biomarkers identified in the systematic review that are available

INTERNATIONAL NEUROBLASTOMA RISK GROUP TASK FORCE PROJECT PROPOSAL

Integrated Cohort Phase

- To identify a homogeneous cohort of patients treated in large cooperative trials that is incorporated to the INRGdb with available biomarker data and/or available samples



- To analyse a set of biomarkers identified in the systematic review and/or the INRGdb (3 to 7, approximately) in all patients
- To conduct a multivariate analysis of all biomarkers in this patient cohort to identify the most powerful predictors of poor outcome that could render the patients candidates for experimental therapies

Figure 1: summary flow-chart of the project

2. Hypothesis

An integrated analysis of clinical and biological factors will identify upfront a subgroup (or several subgroups) of high risk neuroblastoma patients whose outcomes are very poor. These patients should be candidates for early access to innovative therapies.

3. Patient Cohort (Eligibility Criteria)

Patients with high risk neuroblastoma as defined by INRGSS

- Any MYCN-amplified tumor
- Stage M, ≥ 18 months old at diagnosis

* During the conduct of the analysis, other high risk characteristics might be considered, such as unfavorable histology (used by COG as a high risk feature) or recently identified molecular features (e.g. genomic

INTERNATIONAL NEUROBLASTOMA RISK GROUP TASK FORCE PROJECT PROPOSAL

amplifications or distal 6q loss, Depuydt JNCI 2018). Where relevant, some biomarker analyses will be conducted in subpopulations such as MYCN amplified or non-amplified patients.

4. Background

The outcome for all children with high risk neuroblastoma remains poor despite multimodal therapy, but outcomes are particularly poor for a subpopulation of children, including those who die early during their disease course. These patients derive minimal benefit from current highly toxic therapies. If these patients were identified at presentation, they could be candidates for experimental therapies much earlier in their disease course maximizing their chances of benefit. Furthermore, identifying this group would provide a focus for biological investigations and would provide accurate information to parents. Although there is no consensus in terminology, this group of patients has been referred to as “ultra-high risk” (UHR) neuroblastoma patients by many, although the term is not uniformly defined and accepted.

To date, many efforts have been undertaken to identify patients with high-risk neuroblastoma who have a worse outcome by using clinical and biological factors and biomarkers, including several -omics platforms.

Some prominent studies identifying prognostic factors in high risk neuroblastoma include:

- 6q loss and amplifications (Depuydt, JNCI 2018)
- Circulating neuroblastoma mRNAs (Viprey, JCO 2014)
- Gene expression classifiers (Formicola, Transl Med 2016)
- Expression signatures (Asgharzedah, JCO 2012)
- Combinations of clinical factors on a prognostic score (Morgenstern, PBC 2018)

Many of these investigations have proposed biological features to identify high risk cases with worse outcome but have not compared them to other potential features and there have been no multivariate analyses within the same dataset. Furthermore, many of the cohorts include patients not treated in a homogeneous manner. Systematic reviews and meta-analyses of prognostic biomarkers have been conducted in multiple other cancer types leading to the identification of the most powerful biomarkers using a methodology with the highest level of evidence (e.g. Chen BMC Cancer 2013 or McCormick Matthews BJC 2015 for esophageal cancer).

Moreover, several initiatives by collaborators in this group have also been launched gathering experts to elaborate a consensus definition for this cohort of patients based in expert opinion. So far, no consensus definition of “UHR” neuroblastoma has been achieved. Challenges have recently been summarized by Morgenstern et al. (manuscript submitted) and include difficulties in selecting the most appropriate endpoint, wide heterogeneity of datasets and markers used, and changes in therapy across time.

In brief, there has been no consensus on: i) how to define this cohort of patients that could be candidates for experimental therapies upfront, particularly with a clear yes/no definition (binary outcome) ii) What is the most powerful approach to identify this cohort of patients at presentation? Is it using a single factor (biological or clinical) or a combination of different subgroups or a combination of different factors in a score or nomogram?

A reasonable benchmark can be those patients with high-risk neuroblastoma who have an overall survival of $\leq 10\%$ at 18 months (end of multimodal treatment), although the degree of poor outcome of the selected

INTERNATIONAL NEUROBLASTOMA RISK GROUP TASK FORCE PROJECT PROPOSAL

group may evolve as the analysis progresses and may depend on the context or purpose for which the cohort is being identified.

For the purpose of identifying this cohort of patients as candidates for experimental therapies, we propose that the identification of this group should not be based on “expert opinion” or “international consensus” but on a systematic development and validation process, as is done to qualify a novel biomarker. The INRG offers an ideal opportunity to host this work as i) it already has data from 22,000 patients; ii) the INRGdb has “inbuilt bioinformatics” capacity through the INRG Data Commons and the Bionimbus Protected Data Cloud; iii) there is a robust Governance process and iv) a great deal is already known about the data due to the extensive statistical analyses that have been performed to date. On the other hand, some biological, biomarker and genomic features are not currently included in the INRGdb. This investigation would build on the existing international collaboration to develop prognostic classification based on incorporating newer biomarkers including a recent position paper (Morgenstern, submitted), several INRG analyses on biological (Schleiermacher and Maris) and clinical (London, Moreno, Morgenstern) factors, and incorporating datapoints from other studies within COG, SIOPEN or other study groups.

Of note, this study proposal aims to analyze prognostic factors that are present at the time of diagnosis of high risk neuroblastoma. Prognostic biomarkers of poor response to induction treatment have also been proposed, such as minimal residual disease-type markers such as circulating mRNAs or MIBG scoring methods, but, for comparability, these will be outside this study proposal.

Once a definition of a UHR cohort as candidates for experimental therapies has been validated through the proposed process, it can be tested prospectively and eventually implemented in the next generation of high-risk trials.

5. Significance

Currently most patients with high risk neuroblastoma receive the same frontline treatment regardless of their clinical or biological characteristics, with few exceptions, such as patients whose tumors harbor ALK aberrations receiving ALK inhibitors in the new COG trial or poor responders to induction chemotherapy receiving more intensive therapy in the SIOPEN high risk trials.

Still, many neuroblastoma patients will experience relapse and succumb from their disease. Many patients, particularly those with early relapses, derive minimal benefit from current intensive multimodal frontline therapy.

If this multi-step project is successfully completed, a subgroup (or subgroups) of patients with particularly poor outcome will be identified upfront at the time of diagnosis. This will not only provide useful information for clinicians and families, but will make these patients candidates to access novel drugs within early clinical trials at much earlier timepoints, without waiting for their disease to relapse or become refractory to treatment.

A recent example is seen in children with diffuse intrinsic pontine glioma (DIPG). Traditionally these patients were only enrolled to early phase trials at the time of relapse. The low overall survival of these patients (<10% at 2 years) (Cohen NeuroOncol 2011, Hoffman JCO 2018) has motivated a change in this paradigm, and now most early phase clinical trials designed for DIPG include patients at diagnosis, including upfront radiotherapy. This model could potentially be applied to UHR neuroblastoma patients in the future.

6. Proposal description

Systematic review Phase

INTERNATIONAL NEUROBLASTOMA RISK GROUP TASK FORCE PROJECT PROPOSAL

During this part, a systematic review of all published studies analyzing clinical and biological prognostic factors in high risk neuroblastoma will be conducted. The review will follow current guidelines for systematic reviews of prognostic biomarker studies.

This will serve 1) to identify all the potential definitions of this poor outcome cohort that have been used (based on OS, EFS, longer/shorter time frames) and 2) to identify all the methods/technologies that have been used to identify this worse prognosis group (-omics tools, biological or clinical factors, etc.)

If the quality of the studies is sufficient, a meta-analysis will be conducted, and forest plots generated, for a more robust evaluation of the performance of each biomarker.

INRGdb Phase

During this phase, the following steps will be completed:

- The performance of the different definitions of this poor prognosis cohort found in the systematic review will be evaluated in the INRGdb.
- We will identify which biomarkers are already (or close to be) available in the INRGdb (biological, genomic), or would be available by linking to other databases (e.g. TARGET or R2)
- The performance of the different biomarkers that are available in the INRGdb will be evaluated conducting multivariate analysis.
- A final stage of this phase will include expert consensus work to agree on 1) the best definition to identify this cohort of patients with worse outcome that would be candidates to experimental therapies and 2) which biomarkers from the literature review or the INRGdb perform best and should be selected for testing in the integrated analysis. These decisions will be largely supported by evidence generated by the systematic review and the analysis of INRGdb.

Integrated Cohort Phase

- A cohort of patients with HRNBL that has received homogeneous therapy and have available biomarker data (or available samples) will be identified. Most likely, this will come from recent clinical trial cohorts (such as ANBL0532 or SIOPEN HBNL1), and will be achieved through collaboration with the specific groups/labs.
- In this cohort, all biomarker analyses will be performed for all patients.
- In this cohort, a combined integrated comprehensive analysis of all clinical and biological factors will be performed to identify the patient population(s) associated with worse outcome. If several characteristics or subpopulations are present, a nomogram will be developed for clinical use.
- The final step will be to validate the characteristic/score/nomogram in independent cohorts (new trial cohorts that are added to INRGdb, prospective collection in future frontline trials or evaluation in the wider INRGdb cohort of HRNBL patients) and validate its performance as a diagnostic/stratification test: sensitivity, specificity, positive and negative predictive values and likelihood ratios.

7. Data Requested

- For the systematic review of the literature, no data are requested from INRGdb.
- For the INRGdb Phase, all patients with high risk neuroblastoma present in the INRGdb from 2000 onwards will be analysed. This data will include newly added genomic information and potential links with other databases.
- For the Integrated Cohort, two to three trial datasets will be identified (most likely ANBL0532 and HRNBL1). In collaboration with the relevant collaborators from COG and SIOPEN trials and respective laboratories, availability of biomarker data and tumor samples will be assessed.

INTERNATIONAL NEUROBLASTOMA RISK GROUP
TASK FORCE
PROJECT PROPOSAL

Appendix 1: Systematic Review Methodology

- 1) **Literature search:** aimed to identify all primary literature examining prognostic markers in neuroblastoma. A search strategy will be developed. First suggestion: neuroblast* AND (prognos* OR surviv* OR mortal*) AND (marke* OR biomark*). Second suggestion: neuroblast* AND (prognosis OR event free survival OR survival OR mortality) AND (marke* OR biomark*)\
 - a. Search will be conducted in PubMed without time limits for articles in English language
 - b. Existing systematic reviews and reference lists will be searched
 - c. Existing Guidelines for Systematic Reviews of Biomarkers will be followed: PRISMA for reporting meta-analyses and MOOSE (Meta-analyses Of Observational Studies in Epidemiology, Stroup JAMA 2000) for reporting prognostic factors. \
- 2) **Eligibility.** Studies published from 2000 to 2018 (both included) analyzing prognostic markers in neuroblastoma associated with survival outcomes will be identified. Only those studies that specifically analyze high-risk neuroblastoma patients or those that include a separate analysis for high risk cases will be selected for the systematic review. Studies with <50 cases will not be included. Patient cohorts should include patients treated from 1995 onwards and have received conventional high risk neuroblastoma treatment including autologous stem cell transplant. Quality of evidence will be evaluated using the ASCO Level of Evidence (LOE) Scale for Biomarker Research (A, B, C and D)
- 3) **Data collection:** for the relevant articles, variables will include: author, type of biomarker, year of publication, number of cases, outcome endpoint of choice (OS, EFS... primary and secondary), treatment received (including high dose chemotherapy and anti-GD2 therapy), date of treatment (at 5 year intervals from 1995), uni or multivariate analyses, hazard ratios or other endpoints to assess biomarker performance (95% CI) and p values.
- 4) **Statistical analysis**
 - Both uni and multivariate results will be considered
 - Biomarkers will be grouped according to the following categories: clinical biomarkers, genomic biomarkers and expression biomarkers,
 - If quality of the data permits, meta-analysis will be carried out for all markers with a statistically significant association ($p < 0,05$)
 - Where possible, analyses of study heterogeneity and bias will be conducted
 - Figures for individual studies and broad categories will be generated, including forest plots
- 5) **Outcomes.** The systematic review and meta-analysis will seek two objectives
 - To identify and collect all definitions of worse prognosis ("UHR" neuroblastoma) that have been used in the literature (DFS, EFS, OS, binary endpoints...) for further testing in INRGdb.
 - To identify those biomarkers with the highest hazard ratios (or equivalent) for further testing in the INRGdb.
- 6) The report will follow PRISMA guidelines (methods & results section)
- 7) Upon completion of the systematic review and meta-analysis, the results will be presented at an international conference and a publication prepared. The outputs of the systematic review will feed into the overarching INRG project as described above.