EVALUATION OF BIOMARKERS IN RELATION TO RECURRENCE RATE IN CHILDHOOD EPENDYMOMA

FINAL REPORT

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1. INTRODUCTION

Ependymomas represent 10% of childhood brain tumors and 30% of those in children less than three years of age; approximately 35 new cases are diagnosed each year in Canada. *Childhood ependymomas have very high moribidity and mortality rates, and there are currently no effective tools for prognosis or risk assessment.* In this proposal, we have developed a unique resource for this rare and serious tumor by developing a Canadian clinical database and tissue microarray (TMA) bank. The clinical data and TMA bank will become a valuable resource for future studies that address mechanisms, therapies and outcomes of childhood ependymoma. Data from the study will provide several benefits, including: better understanding of the biology of ependymoma; the basis for a new risk stratification system for childhood ependymoma; and building blocks for prospective Canadian biological and clinical trials on ependymoma. We anticipate that our work will eventually lead to the development of Canadian guidelines for therapy of this childhood cancer.

Using ependymoma tissues obtained at surgery, we had two primary goals:

1) To build Canada's only pediatric ependymoma tumor tissue microarray (TMA) with linking to a clinical database; this will provide a valuable resource to develop an improved classification system that includes biological biomarkers;
2) To examine ependymoma for activation of the EGFR/Her-2/Her-4/Akt/YB-1 pathway and relate the expression profile to clinical risk factors, and outcome.

In the proposed project, we will assess *clinical factors, histological features and whether the Her-2 pathway is activated in 240 children diagnosed with ependymoma*. All of the signal transduction proteins we propose to evaluate in our population have been shown to be of prognostic significance in cohorts of fewer than 120 patients with breast cancer, colorectal tumors or medulloblastoma, suggesting the feasibility of evaluating these factors and showing significance in our Canadian population. Thus, there was a strong rationale for building a Canadian unselected population-based ependymoma tissue microarray (TMA) bank.

1.1 Approvals Status

The initial approval for each of the 12 participating sites is shown in Table 1 below:

Table 1. Study Approvals

Study Site	Date of Initial Approval
BC Children's Hospital – Vancouver	May 2006
Alberta Children's Hospital –Calgary	March 2008
Stollery Children's Hospital – Edmonton	March 2008
IWK Health Centre – Halifax	May 2008
The Hospital for Sick Children –Toronto	January 2008
Children's Hospital of Western Ontario – London	May 2008
McMaster University Health Sciences Center – Hamilton	December 2006
Children's Hospital of Eastern Ontario – Ottawa	December 2007
Kingston General Hospital – Kingston	November 2007
Montreal Children's Hospital – Montreal	June 2009
Manitoba Cancer Center – Manitoba	November 2007
Centre Hospitalier Universitaire Sainte-Justine	September 2009

Centre Hospitalier Universitaire de Québec – Québec has cancelled its participation in the study before they obtained ethics approval.

All of the participating sites are renewing their ethics approvals with their local Institutional Review boards on an annual basis.

1.2 Agreements Status

Agreements have been fully signed with all of the 12 participating sites. The last agreement was fully signed in October 2009.

Centre Hospitalier Universitaire de Québec – Québec has cancelled its participation in the study before signing an agreement.

The agreements have been respected from all sides and there are no issues in that regard.

2. INITIATATION AND DEVELOPMENT OF STUDY AT BRITISH COLUMBIA CHILDREN'S HOSPITAL (BCCH)

The initial step of the study was to identify potential study subjects. All children admitted to BCCH, with a diagnosis of ependymoma before 18 years of age were screened. There were 79 eligible ependymoma patients identified, 49 of whom had Ependymoma specimens available. Comprehensive chart reviews were conducted for all 79 eligible patients and Clinical Data Forms and Clinical Data Follow-up Forms were completed. The clinical data was subsequently compiled onto a main database. The 49 specimen blocks were centrally reviewed and scored by Dr Stephen Yip and Dr Glenda Hendson at BC Children's Hospital and Dr Cynthia Hawkins, Neuropathologist at The Hospital for Sick Children in Toronto, designed the Tissue Microarray. The pathological data was linked to the clinical data and compiled onto the main database. The clinical data and the pathological data were subsequently analyzed in January 2008 and the findings were published in an Abstract (enclosed in Appendix B). An additional group of previously diagnosed 5 patients were identified in 2008 providing a new number of 84 patients with clinical data available. In February 2010, after careful cleaning of the main database, 35 study patients have been removed as ineligible: 31 were found initially diagnosed before 1986, one patient initially treated abroad, one patient had conflicting pathology reports with no pathological specimens available to confirm the diagnosis and two patients were excluded after the pathological review showed presence of tumors other than ependymoma. One patient died on the day of diagnosis and was therefore excluded from the analysis linking the clinical data to molecular biomarkers. The final version of the database includes 48 eligible patients from Vancouver with tumor specimens.

3. EXECUTION OF THE STUDY AT THE OTHER PARTICIPATING CANADIAN CENTRES

Twelve pediatric oncology centres across Canada are participating in this study covering the whole population of British Columbia, Alberta, Manitoba, Ontario, Maritime Provinces and part of the Quebec population.

After the signing of the agreements between BC Children's Hospital and each pediatric oncology centre participating in the study, each site obtained ethics approvals from their local IRB board, identified potential subjects, gathered data, identified specimens and sent all materials to the coordinating centre. The first site completed the on-site activities in July 2008 and the last site completed the on-site activities in January 2010.

The total numbers of estimated patients, specimens, and data completion forms for each of the 12 participating sites are shown in tables 3 and 4 below.

Table 3: Estimated vs. enrolled eligible patients with clinical data.

Site	Estimated	Received
Alberta Children's Hospital (Calgary	10	13
Stollery Children's Hospital (Edmonton)	10	11
IWK Halifax	10	23
The Hospital for Sick Children (Toronto)	87	89
Children's Hospital of Western Ontario (London)	10	5
McMaster University Health Sciences Center (Hamilton)	10	2
Children's Hospital of Eastern Ontario (Ottawa)	15	12
Kingston General Hospital	10	5
Montreal Children's Hospital	23	6
Manitoba Cancer Center	10	16
CHU Sainte-Justine (Montreal)	10	32
UBC (Vancouver)	49	49
Total: 263 Identified duplicates: 7 Corrected Total:	**254**	256

Table 4: Number of eligible patients with pathological specimen(s) and cores on TMA.

Site	Received	Cores on TMA
Alberta Children's Hospital (Calgary	12	>90%
Stollery Children's Hospital (Edmonton)	11	>90%
IWK Halifax	23	>90%
The Hospital for Sick Children (Toronto)	70	~50%
Children's Hospital of Western Ontario (London)	4	>90%
McMaster University Health Sciences Center (Hamilton)	1	>90%
Children's Hospital of Eastern Ontario (Ottawa)	12	>90%
Kingston General Hospital	2	>90%
Montreal Children's Hospital	6	>90%
Manitoba Cancer Center	7	>90%
CHU Sainte-Justine (Montreal)	18	>90%
UBC (Vancouver)	47	>90%
Total: 218 Identified duplicates: 5 Corrected Total:	**213**	

In summary, all 12 sites have completed the primary on-site activities: identifying study patients, comprehensive chart review, collecting data and completing the provided clinical data forms as well as identifying and shipping the available pathological specimens. All these activities have been completed by February 2010. Long-term follow up was conducted at sites that provided initial data in 2008 and early 2009.

Concurrently with the receipt of data collection forms, the available data has been entered in the main database. Data entry was completed in February 2010. We have received data for 330 patients and pathological specimens for 238 patients from all participating centres. After careful search to identify all ineligible and duplicate patients, we completed the database with 256 patients of which 213 or 83% have at least one pathological sample available for the study.

The pathology specimen collection, review and TMA building, as well as immunostaining has been done in two instances. Following the trend in receiving the pathological samples, the first TMA was built as a pilot TMA and staining for the same was performed in January 2008. The pathological and immunostain data from the pilot TMA were then linked to the clinical data and compiled onto the main database. A few conference proceedings and abstracts were published from the first results as listed in the Appendix B. The collection of the rest of the pathological samples took longer time with the last pathological sample being received in November 2009. The pathological review of all of the remaining cases was completed in February 2010. The available specimen blocks were reviewed and scored by Dr Christopher Dunham, Neuropathologist at BC Children's Hospital and Dr Steven Yip, Neuropathologist at BC Cancer Agency in Vancouver. In April to May 2010, the TMA block was prepared. Further immunostaining was performed in November 2010 and the scoring of the immunostain is ongoing. The full pathological data and immunostaining will be linked to the clinical data and compiled onto the main database immediately after the immunostain scoring is complete.

The overall timeline of the study has changed from the initial proposal as it took much longer for some sites to obtain ethics approvals, and for some sites to finalize the sub-site agreements. The late ethics approval was due to different policies of the local ethics boards. Another issue that consumed additional time was the need of translating and compiling informed consent in French at the two Québec sites. Some sites have faced difficulties in obtaining data and/or pathological specimens. The Québec sites had to obtain informed consent of the subjects to share data and pathological specimens, and that additionally prolonged the study implementation.

After the immunostain scoring is done, and linkage of this data to the clinical data is complete, the last phase of analysis of data of the project remains to be done. We expect that this phase will be completed in the first quarter of 2010. The planned vs. actual timeline for the overall study is given in Table 5 below.

Table 5: Study timeline, planned vs. actual duration of study activities

ACTION	Planned = P, Actual = A	YEAR 1						YEAR 2						YEAR 3						YEAR 4					
		Oct-07	Dec-07	Feb-08	Apr-08	Jun-08	Aug-08	Oct-08	Dec-08	Feb-09	Apr-09	Jun-09	Aug-09	Oct-09	Dec-09	Feb-10	Apr-10	Jun-10	Aug-10	Oct-10	Dec-10	Feb-11	Apr-11	Jun-11	Aug-11
Ethics Approval	P																								
	A																								
Identify Patients & Pathology Specimens	P																								
	A																								
Review Pathology & Build Tissue Microarray	P																								
	A																								
Perform & Score the Immunostaining	P																								
	A																								
Review & Analyze Clinical Data	P																								
	A																								
Analyze, Correlate Clinical & Biomarker Data & Results of Study	P																								
	A																								

4. DEVELOPMENT OF STUDY DOCUMENTATION

4.1. Data Collection Forms

Clinical data collection forms (DCFs), as well as a Clinical Data Follow-up form have been created, updated and used by all study sites for the study purposes. The Hospital for Sick Children from Toronto is the only site that didn't use the Clinical Data Form provided but sent the data compiled in a spreadsheet. That data was then used and entered in the main database.

A List of the data collection forms and their content is given below:

1. **Clinical Data Collection Form**

   Includes:
   a. Demographic Information
   b. Tumour Characteristics
   c. Surgical Interventions
   d. Adjuvant Therapy at Diagnosis
   e. Chemotherapy
   f. 1st Progression
   g. Subsequent Therapy at 1st Progression
   h. Status at last F/U

2. **One Year Follow-Up Form**

   Consists of:
   a. Relapse
   b. New Complications of Therapy
   c. Update on Neurocognitive Status

The DCFs and the 1 Year Follow-up forms have been already submitted in the previous annual reports. They remain unchanged.

4.2 Study Procedures and Study Update Log

A summary of study procedures was created to assist participating centers with the conduct of the study. The national study coordinator manages a study update log, an excel spreadsheet document to keep track and update the status of study activities at the sub-sites including data collection, pathological specimens identification and shipping, as well as the payments processing.

The study procedures and update log have been already submitted in the previous annual reports. They remain unchanged.

4.3 Standard Operating Procedures (SOPs)

A document outlining the standard operating procedures (SOPs) for the main study coordinator was created and is being kept updated.

The SOPs have been already submitted in the previous annual reports. They were updated accordingly, to represent the staff change at our site.

APPENDIX A – Up to Date Financial Report

APPENDIX B – List of Abstracts and Publications