Burkitt Lymphoma Genome Sequencing Project
Public-Private Partnership Proposal to

PROSPECTIVE DONOR NAME

DRAFT 11/15/13

Quick Summary

The Burkitt Lymphoma Genome Sequencing Project (BLGSP) was initiated in 2010 by the National Cancer Institute (NCI) as a public-private partnership with the Foundation for Burkitt Lymphoma Research (FFBLR), coordinated by the Foundation for the National Institutes of Health (FNIH). The goal of the partnership is to develop a genomic databank for Burkitt lymphoma (BL). BLGSP will compile genetic changes present in BL tumors, analyze the data to identify diagnostic, prognostic, or therapeutic markers or targets, and publish the results**.** The goal of the project is to identify potential genetic changes in patients with BL that could lead to better prevention, detection and treatment of the cancer.

The BLGSP is projected to take up to five years to complete and includes the following phases:

- Phase I: Document Development, Investigator Recruitment, and Tissue Accrual (24-36 months). This phase is underway, with funding from the FFBLR and in-kind resources and infrastructure support from the NCI. Phase I is ongoing through 2014 and is described in the BLGSP Study Protocol, which is available upon request.
- Phase II: Genome Sequencing and Analysis. This phase will start during Phase I as soon as a critical number of cases (12-20) are accrued and will end less than 1 year after the last cases have been collected.
- Phase III: Validation of findings in a new population cohort (6 months).
- Phase IV: Publication (3-6 months).

Further private-sector involvement, both scientific and financial, is essential to the success of this project. NCI intends to carry out Phases III-IV through an expanded public-private partnership, including FFBLR as Founding Partner and a diverse group of additional corporate, foundation, individual, and non-profit stakeholders.

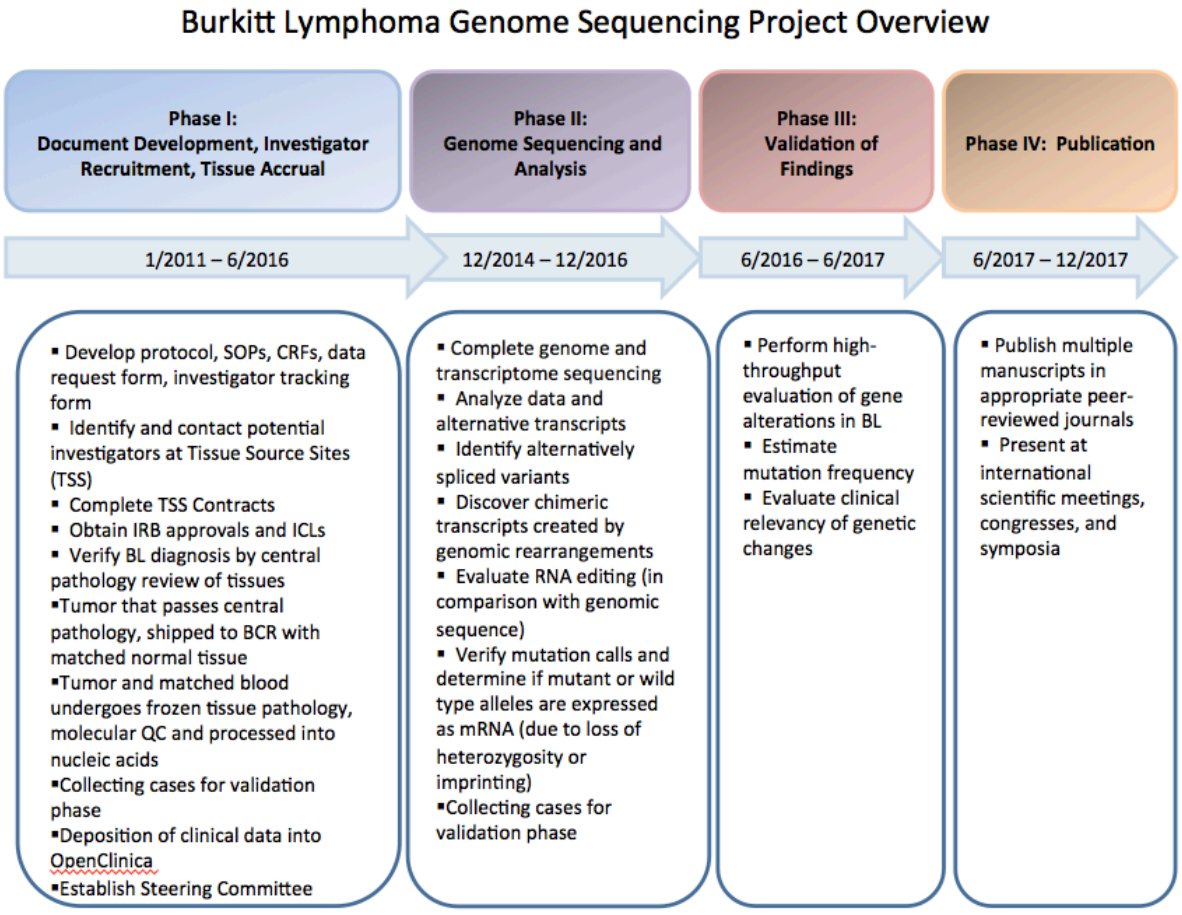
The Foundation for Burkitt Lymphoma Research and other private-sector donors have already committed over $1,100,000 to BLGSP, with the Foundation for Burkitt Lymphoma Research as lead partner. In Phase III, the anticipated costs will be between $800,000 and $1.2 million to collect samples and data for the validation cohort. NCI will continue to cover staffing and infrastructure costs, project management including tissue accrual and sequencing contracts, sequencing of the discovery set samples, the data

repository and data portal as part of the Office of Cancer Genomics (OCG) and the Data Coordinating Center (DCC).

The FNIH, an independent, nonprofit organization established by the U.S. Congress to support the mission of the NIH, now seeks the $1 million needed to fund accrual for Phase III of the project. This private sector investment will leverage the value of NCI's significant, multi-year staffing and resource commitment—in effect sharing the risk of this research investment across multiple parties and yielding results well beyond what either the public- or private-sector would be able to achieve alone.

Building on previous experience with major partnerships, and to encourage and facilitate private partner involvement, the FNIH will establish a BLGSP External Scientific Board (ESB), where funding partners can engage scientifically with each other and with NCI project leadership to share information and updates, express viewpoints, address challenges, share expertise, and develop common perspectives on issues relating to the BLGSP.

The table below describes the four Phases of the project and the time line and deliverables for each Phase. The project phases are discussed and summarized in the Project Overview section of this document, beginning on page 4.



Further details of the project and partnership opportunity are given below; the BLGSP study protocol is available upon request.

About Burkitt Lymphoma

Burkitt lymphoma, first described by Dr. Denis Parsons Burkitt in 1956, is an uncommon type of non-Hodgkin lymphoma which often but not exclusively affects children. It is a highly aggressive type of B-cell lymphoma, often involving body parts other than lymph nodes. The disease is associated with a chromosomal translocation of the *MYC* gene. The tumor consists of sheets of lymphoid cells of similar size and shape with high levels of proliferation and cell death. A classical hallmark of Burkitt lymphoma is the "starry sky" appearance of the tumor when visualized under a low-power microscope.

Currently Burkitt lymphoma is divided into three main clinical variants:

1. Endemic: Occurring mainly in equatorial Africa, where Burkitt lymphoma was first described, this variant is the most common malignancy in children. In 95% of cases the children are infected with Epstein-Barr virus, which is presumably key to the etiology of this cancer. The disease is more prevalent in children than adults and characteristically involves the jaw or other facial bone, and may involve the distal ileum, cecum, ovaries, kidney or the breast.
2. Sporadic: Found outside of Africa, this variant is usually not associated with Epstein-Barr virus infection, but has morphological and molecular features in common with endemic Burkitt lymphoma. The ileocecal region is the most common site of involvement.
3. Immunodeficiency-associated: This variant is associated with HIV infection and can accompany the initial manifestations of AIDS. It can also occur in post-transplant patients who are taking immunosuppressive drugs.

Current chemotherapy regimens are effective in approximately 40 to 90% of patients, depending on age, stage of the disease, treatment regimen, and site of the treatment facility. Hence, new treatments are needed to improve the efficacy of current regimens and to potentially substitute less toxic agents for the high intensity chemotherapeutic drugs currently given.

Project Significance

The defining feature of Burkitt lymphoma, the *MYC* translocation, was discovered in 1983. Since then, little progress has been made in understanding the molecular pathogenesis of the disease. MYC is itself insufficient to transform primary cells and actually induces apoptosis (cell death) unless growth factors are provided or cooperating oncogenes are activated. We currently have little insight into the possible involvement of kinases and signaling pathways that might promote the high rate of proliferation that characterizes these tumors. Loss of p53 function by mutation or by inactivation of INK4a/ARF may contribute to cell survival in a fraction of cases, but aside from this, mechanisms that prevent apoptosis of Burkitt lymphoma cells have yet to be discovered. What is needed, therefore, is a comprehensive effort to discover the full array of genetic aberrations associated with Burkitt lymphoma using high-throughput methods.

Several major unmet needs in the clinical management of Burkitt lymphoma require urgent attention.

- First, the current standard treatment of high-dose chemotherapy is ineffective in roughly 20% of cases, and is associated with therapy-induced toxicity and death due to its intensity. As a result, patients with Burkitt lymphoma over the age of 70 are ineligible for this regimen and receive lower doses of chemotherapy, which is less effective.
- Second, young individuals exposed to these intensive regimens face an increased risk of secondary malignancies years later.

- Finally, many children with endemic Burkitt lymphoma in Africa do not receive these curative chemotherapy regimens because of the lack of hospital support for the management of side effects and post-treatment infections. Many of these children will die of their disease.

Thus, new approaches to the therapy of Burkitt lymphoma based on an understanding of its molecular pathogenesis and the regulatory pathways that it utilizes for proliferation and survival are essential.

Burkitt lymphoma is derived from the germinal center stage of B cell differentiation, and as such expresses a mutating enzyme, activation-induced cytosine deaminase (AID). AID is likely involved in generating the breakpoints that lead to *MYC* translocations in sporadic Burkitt lymphoma and almost certainly targets many cellular genes for mutation. Hence, Burkitt lymphoma may utilize AID-generated mutations to modulate oncogene and tumor suppressor pathways. This Burkitt lymphoma comprehensive genome characterization project will shed considerable light on the oncogenic capacity of AID and identify other potential disease-causing candidates.

An additional important question is whether genetic alterations in the Burkitt lymphoma genome may influence the symbiotic relationship with the Epstein-Barr virus in the endemic variant, and in a proportion of the sporadic and immunodeficiency-related cases. EBV viral proteins can mimic Notch signaling (EBNA2), CD10 signaling (LMP1), and B cell receptor signaling (LMP2). It may be necessary for the Burkitt lymphomas to acquire mutations in these pathways to shape the cellular response to these virally-derived signals.

Many key clinical questions may be illuminated by knowledge of genomic abnormalities in Burkitt lymphoma. Why do approximately 10 to 60% of patients treated with potentially curative chemotherapy nonetheless succumb to their disease? How do the genomic alterations in the Burkitt lymphoma clinical variants (endemic, sporadic, immunodeficiency-related) differ, and how do these differences affect their response to therapy? What new therapeutic options may be available to augment or supplant high-dose chemotherapy?

The BLGSP holds much promise for answering these questions and uncovering new insights into the mechanisms of Burkitt lymphoma that may lead to prevention strategies and more effective treatments for those living with this disease.

Project Overview

The Burkitt Lymphoma Genome Sequencing Project (BLGSP) will lay the groundwork for this research.

A major objective of the BLGSP is to generate complete sequence of genomes and transcriptomes (mRNA and miRNA) from case-matched tumor-normal pairs from patients with BL. The study will include adult and pediatric patients with sporadic Burkitt lymphoma, as well as people with endemic and HIV-positive sporadic BL. Clinical data on each is a major requirement for this project so that associations between clinical parameters and genetic abnormalities can be discovered. Through collaborative efforts with researchers working in East Africa, *the BLGSP will soon meet its tissue accrual goals for patients with endemic BL.* Obtaining tissues from patients with sporadic and HIV-associated BL is a high priority for BLGSP, and it is expected that tissue accrual for the discovery phase will be completed by December 2014.

The NCI, through the Office of Cancer Genomics (OCG), provides the administrative and analytical infrastructure needed for BLGSP, including standard operating procedures (SOPs) (available at

http://ocg.cancer.gov//sites/default/files/OCG_SOP_Manual.pdf) developed by OCG that were adapted from others used in large-scale molecular characterization projects, as well as the BLGSP study protocol. Participating institutions, known as BLGSP Tissue Source Sites (TSS) use the SOPs to prospectively collect tissue (tumors and patient-matched normal, *e.g.* blood) from well-annotated cases of Burkitt lymphoma.

NCI project leadership is aware of and sensitive to the issues surrounding the informed consent in non-U.S. countries that relate to children and has made available template informed consent as part of the web-based SOPs that can be adopted as appropriate. In addition, the tissue providers' institutions need to acknowledge that the project protocol and its goals are in line with the informed consent approval.

NCI proposes to initiate sequencing after a critical number of cases (12-20) are accrued. Analysis of the sequence will provide information regarding tumor-associated chromosomal aberrations (e.g. deletions, amplifications, translocation, somatic mutations, and gene expression alterations), as well as identification of chimeric or alternatively spliced transcripts.

The first phase of this project, already funded by the FFBLR, is expected to be complete in December 2014. Phases II-IV will take approximately three additional years. This public-private partnership will partially fund Phases III and IV.

Data generated from the BLGSP will be submitted to publicly accessible databases developed for other large-scale cancer sequencing projects, such as Therapeutically Applicable Research to Generate Effective Treatments (TARGET) (http://ocg.cancer.gov/programs/target) and the Cancer Genome Characterization Initiative (CGCI) (http://ocg.cancer.gov/programs/cgci).

The project has a steering committee whose membership includes members of the FFBLR's scientific advisory board and the FFBLR's executive director, a representative from the contractor(s) performing the sequencing and tissue processing, members of the pathology review board and representatives from OCG.

Phases II-IV of the project are described below.

Phase II: Genome Sequencing and Analysis

Sequencing is expected to begin as soon as a critical number of cases (12-20) are accrued during Phase I. It will include whole genome as well as transcriptome (messenger/micro-RNA) sequencing.

Analysis of the genomic DNA sequence will provide information regarding single-nucleotide polymorphisms (SNPs) for loss of heterozygosity, somatic mutations, genomic rearrangements and copy number. Messenger and micro-RNA sequencing informs on the digital gene expression profiling, identification of alternative spliced variants, discovery of fusion transcripts created by genomic rearrangements, RNA editing (in comparison with the genomic sequence), confirmation of mutation calls, and of mutant or wild type alleles expressed as mRNA (either due to loss of heterozygosity or imprinting).

The NCI-managed project team has developed an analytical protocol for the data generated, including:

- Analysis of the copy number alterations with integration with digital gene expression
- Identification of somatic mutations
- Identification of translocations

The aim is to identify recurrent genetic alterations within Burkitt lymphoma as a whole and within the clinical variant groups. Genetic changes identified through BLGSP will be analyzed in an integrated manner,

focusing on the group of alterations as a whole, rather than individual genes. This method improves the power of the analysis and identification of the drivers of tumor initiation and progression.

Phase III: Validation of Findings in a New Population Cohort
The findings discovered in Phase II will be validated via high-throughput evaluation of gene alterations in a separate BL cohort as a whole, and within the BL variants. Validation studies performed in a new cohort, collected during Phase I, will allow for mutations uncovered in Phase II to be verified as true and increase the power to estimate the frequency with which they occur. To have the statistical power necessary to determine which clinical variants of BL are driven by specific molecular pathways, a large validation cohort representing each of the variants will be needed. Moreover, this validation phase will enable evaluation of the clinical relevancy of the genetic changes found.

Phase IV: Publication
The tissue contributors will be co-authors on the first manuscript and may collaborate in the future on other publications.

The data that are generated will be submitted to publicly accessible databases, including the NCI's Data Coordinating Center (DCC), which allows easy access to all data generated as well as analytical tools developed for other large-scale sequencing projects (*e.g.* TARGET and CGCI). The sequence generated by BLGSP will be submitted to the National Center for Biotechnology Information's Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra) as well as CGHub (https://cghub.ucsc.edu/). The NIH has developed protocols that the submission of the genome and transcriptome sequences to these protected databases ensures that the privacy and confidentiality of the participants is maintained. The BLGSP agrees to abide by the data release policy formulated for the HIV+ Tumor Molecular Characterization Project (HTMCP), located at http://ocg.cancer.gov/sites/default/files/Data_Release_Policy_HTMCP_091713.pdf Every effort will be made to rapidly publish the first manuscript describing the data and including a high-level analysis.

The Opportunity for Private Sector Involvement

The FNIH now seeks private-sector support to provide $1 million needed to fund Phase III of the project. This private sector investment will leverage the value of the tissue accrual efforts already underway, together with NCI's significant, multi-year staffing and resource commitment—in effect sharing the risk of this research investment across multiple parties and yielding results well beyond what either the public- or private-sector would be able to achieve alone.

The Foundation for Burkitt Lymphoma Research, having already provided more than $800,000 in support of the project, is the founding private partner of the Burkitt Lymphoma Genome Sequencing Project. Other private-sector donations, to date, have amounted to nearly $400,000.

As partners, all private-sector entities (companies, private foundations, and nonprofit organizations) that commit to funding the project through a charitable contribution to the Foundation for NIH will be involved in the BLGSP as members of an **External Scientific Board (ESB**). Convened by the Foundation for NIH as a neutral third-party, the ESB provides an independent, open, and pre-competitive forum through which partners can provide scientific input into the BLGSP on project-wide issues including but not limited to:

- Review of the state of the project

- Case accrual
- Validation
- The impact of Burkitt lymphoma in different parts of the world and whether this project is addressing it adequately.

It is anticipated that the ESB will meet in person once each year, and via teleconference annually or more frequently as needed. Face-to-face meetings will be held in conjunction with other relevant meetings when possible.

ESB Membership

The ESB will include representation from all project partners—private funders, federal partners, and Principal Investigators representing the Tissue Sample Sites in order to ensure the free exchange of scientific information and ideas among these groups. Specifically, it is expected to consist of:

- One representative of each new private sector partner organization (representatives must have appropriate scientific expertise; additional scientific experts from partner companies may attend meetings and participate in discussions with prior approval)
- Two representatives from the founding private partner, the Foundation for Burkitt Lymphoma Research (Drs. Jean Paul Martin, Founder, and John Irvin, Executive Director)
- Principal Investigators representing the Tissue Sample Sites
- Two NCI representatives

The ESB will be led by a Chair who represents the private sector. This position will rotate as agreed by the private-sector partners, who will also determine how the seat will be filled.

The Foundation for NIH will also collaborate with private-sector partners to ensure recognition on press releases and other printed materials related to the project, in the Foundation for the National Institutes of Health annual report, on its web site, www.fnih.org, and by other means and on other materials as appropriate. The Foundation for NIH and NCI hope to welcome a broad group of private-sector representatives as partners in this effort.

Funding Request and Summary

The Foundation for the NIH invites PROSPECTIVE DONOR to participate in supporting the BLGSP by making a tax deductible, charitable contribution in the amount of $AMOUNT to the initiative. PROSPECTIVE DONOR's contribution will be payable in a single payment or, if preferable to COMPANY, made via three annual installments beginning in 2013.

As a financial and scientific contributor to the BLGSP, PROSPECTIVE DONOR will play an important role in generating knowledge that can spur additional research, identify and hasten the testing of more effective treatments for disease, and lead to significant improvements in public health.

About the Foundation for Burkitt Lymphoma Research

The Foundation for Burkitt Lymphoma Research (FFBLR) was established in Geneva in late 2009 by Dr. Jean Paul and Dr. Marie-Reine Martin, in memory of their 32 year old son Xavier who passed away from a

relapse of Burkitt lymphoma. The mission of the FFBLR is to further the understanding of the pathophysiology and treatment of Burkitt lymphoma. The first strategic activity initiated and partially funded by the FFBLR to impact this mission is the Burkitt Lymphoma Genome Sequencing Project. For further information about FFBLR, please contact:

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About the Foundation for the NIH

The Foundation for the National Institutes of Health (FNIH) is a leader in addressing complex scientific and health issues. Established by the United States Congress to support the mission of the NIH, the FNIH is a not-for-profit 501(c)(3) charitable organization that brings new resources to a broad portfolio of programs that enhance NIH priorities and activities. The many partnerships FNIH forms and fosters—among federal government, corporations, foundations, nonprofit organizations and individuals—support biomedical research, education and training. Donors to FNIH advance scientific discovery, support our next generation's scientists and improve human health. More information on the FNIH is available at www.fnih.org

For further information about the Burkitt Lymphoma Genome Sequencing Project and to discuss this partnership opportunity, please contact:

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