HTMCP Template #101

HIV+ Tumor Molecular Characterization Project (HTMCP)

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Statistician

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1.0 Schema

Tumors to be accrued:

- HIV-Associated Diffuse Large B Cell Lymphoma
- HIV-Associated Non-Small Cell Lung Cancer
- HIV-Associated Cervical Cancer
- HIV-Associated Anal Cancer

Procedures:

- Samples (tissues) to be obtained prior to oncologic treatment (e.g. neo-adjuvant therapy):
    - Tumor tissue biopsy, tissues from surgical resection and/or tumor bone marrow aspirate (for lymphomas)
    - Case matched normal peripheral blood mononuclear cells; buccal cells or adjacent normal tissues. Blood mononuclear cells are purified and frozen
      All tissues must be snap frozen

- Tissue block or unstained slides from formalin fixed, paraffin-embedded (FFPE) tissue (tumor and/or adjacent normal) and/or bone marrow biopsy must be available

Sample Distribution:

- Frozen tissues, bone marrow, and/or peripheral blood mononuclear cells will be shipped to British Columbia Genome Science Center, Vancouver, Canada.

- Unstained slides of formalin fixed tissue and/or bone marrow biopsy will be shipped to the appropriate designated central pathology lab.

Data Submission:

- Clinical report forms are submitted to the NCI Data Coordinating Center.

2.0 Background and Rationale

2.1 HIV-Associated Malignancies

HIV infection is associated with a variety of malignancies, including "AIDS-defining cancers" and "non-AIDS-defining cancers" [1]. The AIDS-defining cancers are non-Hodgkin's lymphomas, Kaposi's sarcomas, and cervical cancer. AIDS-defining non Hodgkin's lymphomas are predominantly diffuse large B-cell lymphomas, Burkitt's lymphomas, and less commonly primary effusion lymphomas and plasmoblastic lymphomas. Non-AIDS defining cancers that are increased in prevalence among HIV-1 infected individuals include anal carcinomas, Hodgkin's lymphomas, non-small cell lung cancers, and hepatocellular carcinomas.

The cause for increased prevalence of malignancies in HIV-1 infected individuals is poorly understood, and no systematic molecular characterization of these neoplasias has been reported to date. Many HIV-associated malignancies are also associated with other oncogenic virus infections. These include members of the human papilloma viruses and gamma herpes viruses, including Epstein-Barr virus and Kaposi's sarcoma herpes virus (KSHV), however not all AIDS associated malignancies have been linked to such co-infections. Viruses are associated with a variety of malignant and pre-malignant conditions [2]. Human papilloma viruses are the cause of almost all anogenital carcinomas, and approximately 50% of oral malignancies [3, 4]. Epstein-Barr virus is associated with Burkitt's lymphoma, nasopharyngeal and gastric carcinomas, NK/T cell lymphomas, AIDS lymphomas, Hodgkin's lymphomas, post-transplant lymphoma, and pediatric AIDS-associated leiomyosarcomas [5]. KSHV (human herpes virus 8, HHV8) is associated with Kaposi's sarcoma, primary effusion lymphomas, and multicentric Castleman's disease [6]. Human T-cell leukemia virus (HTLV) type 1 causes adult T-cell leukemia and HTLV-associated myelopathy, as well as pneumopathy, uveitis, and immunosuppressive conditions [7]. A recently discovered polyoma virus, Merkel's carcinoma virus, is associated with the majority of cases of Merkel's neuroendocrine skin malignancies. Hepatitis viruses type B (HBV) and C (HCV) are associated with hepatocellular carcinoma, and HCV is also associated with splenic marginal zone lymphomas. Another recently identified virus, xenotropic murine leukemia-related virus (XMRV) may be associated with human prostate malignancy and chronic fatigue syndrome, although this remains controversial [8]. Other viruses have been implicated in collagen vascular, hepatobiliary, and other malignancies, but definitive information is currently lacking [9, 10]. These infections may be pathogenic in immunosuppressed individuals as a result of an impaired cell-mediated immune response resulting in chronic and incompletely suppressed infection. Malignancies may also arise from cytokine release from activated T cells induced by HIV infection or other opportunistic infectious agents complicating HIV infection.

HIV-1 and -2 are associated with immunodeficiency, which predisposes individuals to infections by opportunistic infectious agents, including oncogenic viruses. HIV-associated immunodeficiency also inhibits anti-tumor mechanisms that result in an increased frequency of a variety of tumors [11, 12]. Thus, HIV-1 infection is associated with markedly increased prevalence in AIDS-defining malignancies, such as Kaposi's sarcoma, non-Hodgkin-s lymphoma, and cervical malignancies, as well as increased prevalence of non-AIDS defining malignancies, including Hodgkin's lymphoma, anal carcinomas, as well as plasma cell neoplasms, hepatocellular malignancies, lung and testicular malignancies. The effects of HIV and other viruses on mechanisms of tumorigenesis remain to be defined, and this information may provide a solid foundation for new therapeutic approaches.

Comprehensive sequencing of genomes and transcriptomes in cancers that arise in HIV-infected individuals through the HIV+ Tumor Molecular Characterization Project (HTMCP, http://cgap.nci.nih.gov/Cancer_Types) may provide a starting point for a systems biology approach towards understanding differences in pathway activation among identical histological subtypes of cancers in immunocompetent and immunodeficient patients. The results obtained should provide important clues to the pathways that either allow tumors to counteract immune surveillance mechanisms or are redundant in the presence of an extrinsic oncogenic influence such as oncogenic viruses.

2.2 Rationale

Rapidly evolving sequencing and informatics tools are substantially diminishing costs of comprehensive characterization of tumor transcriptomes and tumor genomes. These advances have resulted in detailed information on the repertoire of alterations in cancers. Novel approaches of genomic sequencing analyses have provided new tools of pathogens discovery and new information on cellular genetic alterations associated with viral pathogenesis.

The availability of high quality, clinically annotated patient samples is crucial for the study of biologic factors that influence the progression and treatment response of HIV-1 malignancies. Comprehensive genomic sequence of HIV-associated cancers may identify diagnostic or prognostic disease signatures, and recurrent "driver" alterations that may be targets for new therapies. It is also possible that the comparison of transcriptomes and genomes between lymphomas from HIV^+ and HIV^- individuals might identify novel non-human sequences that could potentially suggest the presence of transcripts from hitherto undiscovered oncogenic viral agents.

3.0 Objectives

The primary objective of this HTMCP biological protocol is to support investigation of the hypothesis above by accrual of high quality, clinically annotated tissue from patients with HIV-1 malignancies. This material will be used to study clinical, genetic, and immunologic parameters that might have prognostic significance and/or are involved in the initiation and progression of HIV-1 malignancies in the context of the HTMCP initiative. The project include complete genomic and transcriptomic sequencing of HIV-associated diffuse large B cell lymphomas, lung, cervical and anal cancer and matched normal tissue from the same individuals.

4.0 Eligibility Criteria

1. **Diagnosis**. Patients must have a diagnosis of one of the HIV-associated malignancies aforementioned or clinical findings suggestive of a possible HIV-associated malignancy. Patients that had undergone neo-adjuvant therapy are not eligible for the HTMCP.
2. **Age**. Patients must be ≥ 18 years old.
3. **Informed Consent.** Patients must have signed an IRB-approved informed consent document that permits the use of the samples for genomic-based molecular characterization project(s).

5.0 Sample and Data Acquisition and Processing

Samples will be obtained and processed using protocols developed for HTMCP (HTMCP Standard Operating Procedure Manual).

5.1 Tumor Sample Acquisition

Samples will be obtained from HIV positive patients who had diagnosis of any of the cancers listed in page 3 and will undergo either surgery or biopsy from which sufficient quantity of tissue will available along with case matched blood, buccal cells and/or normal adjacent tissue. Not all samples accrued yield RNA and DNA in sufficient quantities or meet the technical quality criteria (DNA: 80% of molecular weight 10,000 or higher; RNA: RNA Integrity Number (RIN) of seven or higher).

Specifically, this protocol requests:

- Permission to obtain solid tumor tissues donated by the patient at the time of the surgery; OR
- Biopsy tissue from a lymph node or other organ involved with malignancy that remains after the necessary samples are used for optimal medical care of the patient. The sample may be obtained by either surgical biopsy(ies) or needle core biopsies (concurrent additional biopsies taken at the same time as biopsy for pathological diagnosis are acceptable).
- The minimum requirement of tumor tissue amount varies with the cancer type, however, as a general rule, 100 mg of tissue is necessary for the HTMCP. All tissues must be snap frozen in liquid nitrogen within 20 minutes of removal following the established protocol provided in HTMCP SOP #102
- About 4 tablespoons of blood drawn from a vein. If the patient objects to having blood drawn, an alternative is to collect normal tissue by swabbing cells from the inside of their cheeks.
- Tissue block (or in its absence, unstained slides) from FFPE tumor must be submitted for centralized pathology (for lymphoma and lung malignancies, HTMCP SOP #107A and B respectively)
- Permission to collect information from the patient medical records, including age, ethnic background, diagnosis, disease history, medical treatments, surgical pathology, and response to treatments.

5.2 Case-matched Normal Tissue Acquisition

All participants in this study will have a 10 mL sample of peripheral blood drawn by venipuncture or cannulation of an indwelling venous access device. Samples will be placed in sterile EDTA, or sodium citrate or heparin anticoagulant vacutainer tubes, and cryopreserved following the established protocol (HTMCP SOP #103). This blood draw may occur at the same time as a blood draw for routine medical care.

In cases when blood draw is not possible, buccal cells will be collected. Adjacent normal tissue from surgery samples could be collected as well.

5.3 Sample and Data Storage

5.3.1 Sample Identification and Assurance of Anonymity

All biological materials and medical information will be coded in HTMCP. Only the designated gatekeeper at each Institution will keep the code key that matches the project identifying number to the personally identifiable information (http://datacenter.cit.nih.gov/interface/interface241/PIIguide.html; Note: this is applicable in the US, other countries may have different regulatory frames that must be complied with) using procedures in place and approved by the local institution. Researchers, including those who will be working with the patient samples and medical information, will not have access to any of the traditionally used identifying information about the patient. All materials submitted to the HTMCP will be labeled with a project-assigned ID (as described in HTMCP SOP #106).

5.3.2 Storage and Release of Samples and Medical Information

The coded tissue samples will be sent to the Genome Science Center of the British Columbia Cancer Agency (BC-GSC), which is the characterization center for the HTMCP. The samples will be processed there and the molecular analytes extracted from samples will be used for sequencing. Any remaining samples will be stored at the BC-GSC until the end of the project. At the end of the project, any remaining samples will be handled in accordance with the protocol of contributing institution as designated in the disposition form (HTMCP SOP #108).

Data stripped of identifiers, in compliance with the definition specified in the HIPAA Limited Data Set definition (http://hipaa.wisc.edu/ResearchGuide/limiteddatasets.html), will be submitted by the contributing institution to the Data Coordinating Center (DCC). The DCC serves as a central HTMCP project database. The DCC also stores the molecular profiling data generated with the DNA and RNA.

5.4. Sample Shipment

The complete sample sets (tumor and case-matched normal DNA source) will be shipped to the BC-GSC following the procedures explained in HTMCP SOP #104 and 105.

5.5. Research Plan Outline

Samples will be processed and analyzed at the GSC by high-coverage genomic and transcriptomic sequencing. The results will be analyzed will be made between tumor and normal DNA to identify the somatic changes present in the cancer tissues. These alterations include detection of chromosomal changes, such as, but not limited to, amplification (and levels), deletions, loss of heterozygosity, translocations, etc., expression profiling as well as detection of transcripts resulting from translocations and mutations, including single nucleotide variants, insertions, deletions etc. . The results from the tumors of one type will be examined for patterns of common changes, including mutations as a first step to identify the molecular changes that drive the cancer etiology. The alterations will also be analyzed within the context of biological pathways and systems biology.

5.6. Clinical Data Collection

For patients whose samples will become part of HTMCP, clinical information will be collected as described in the clinical report form (for lymphoma lung and cervical malignancies, HTMCP SOP #101A, B and C respectively). These patients will be followed prospectively in order to record the types of treatment given and treatment outcome and toxicity. Follow-up information will include the results of subsequent laboratory and imaging tests, pathology, cytogenetic and molecular diagnostic reports, and records describing the patient's course in the inpatient and outpatient setting. (Note: this enumeration of datapoints is specific for HTMCP project but might not be necessary in the protocol depending on your IRB practices).

5.7. Data Dissemination

- Information (data) from analyses of the coded samples and the coded medical information will be deposited into publicly available databases. These databases will be accessible by the Internet. Medical information and molecular characterization results on the coded samples will be stored in a controlled-access database. The information in this database will be available only to researchers have received approval from the NCI Data Access Committee after their institutions have certified their adherence to patient data protection policies for the project (http://epi.grants.cancer.gov/dac/charter.html).
- Anonymous information from the analyses will be put in a public database, available to anyone on the Internet.

6.0. Financial Compensation/Costs

Patients will not be paid to participate in this project. Tissue samples and the medical information will be used only for research purposes and will not be sold. It is possible that some of the research conducted using the samples or information eventually will lead to the development of new diagnostic tests, new drugs or other commercial products. Should this occur, the patient will not receive any part of the profits generated from such products.

The patient will not incur any expenses from participating in this project.

The chance that the patient will be physically injured as a result of participating in this project is very small. However, if the patient is physically injured as a result of participating in this project, emergency medical treatment for the patient's research-related injury will be provided to the patient at no cost. (Note: this paragraph might not be applicable to your institution, if so, please remove).

7.0. Potential Patient Risks/Benefits

7.1. Potential Benefits of Participating in the Project

The patient should not expect to personally benefit from this research. The main reason the patient may want to participate is to help researchers and health professionals around the world to better understand the causes of cancer and so that they can find better ways to prevent, detect, treat, and cure the disease in the future.

7.2. Potential Risks of Participating in the Project

This project is considered a *minimal risk* protocol

7.2.1 Physical Risks

- If a blood sample is NOT taken, there are no physical risks associated with this project.
- If a blood sample is taken, the physical risks are minimal. Possible risk from blood draw include mild pain, bleeding, bruising, and infection at the site of the needle insertion. Short faint or light-headedness can sometimes occur..

7.2.2. Psychological or Social Risks Associated with Loss of Privacy

Breach of confidentiality is likely the greatest risk of participating in this study. Every effort will be exerted to minimize this risk. There also may be other privacy risks that we have not foreseen. While we believe that the risks to the patient and his/her family are very low, we are unable to tell exactly what all of the risks are.
Despite the extensive security measures employed to protect the identities of patients and their donated tissue specimens, there is a possibility that the identities of patients enrolled in this study could be discovered or linked to genetic sequence data obtained from their tissue specimens. Consequently, it is possible to use this information to link them to the identities of their children, parents, siblings, and other relatives. It may be possible to identify patients as carriers of genetic mutations. It is also possible that there could be violations of the security used to store the codes linking patient's genetic information. In the case of such breach, there could be risks of denial of employment, insurance, etc.

8.0. Project Results

Individual results from this research project will not be given back to the patient or put into the patient's medical records. If research from this project is published in professional journals, there will be no traditionally-used identifying information, such as the patient's name, address, telephone number, or social security number, included in the publications. Some publications from this project will be found at the HTMCP website [http://cgap.nci.nih.gov/Cancer_Types].

9.0. Alternatives to Participating in the Project

The alternative option is not to participate.

9.1 Voluntary Participation

The choice to participate in this research by consenting the use the patient's donated tissues and medical information for the HTMCP project is completely up to the patient. No matter what the patient decides to do, his/her decision will not affect their medical care.

9.2 Withdrawal from the Project

Once the molecular analysis and patient information have been transferred to the DCC, it will not be possible to destroy those data. At the end of the project, unused tissue samples will be destroyed or

returned to the contributing institution as is specified in protocol (HTMCP SOP #108).

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