BIOCYTICS, INC.

RESEARCH STUDY PROTOCOL

TITLE: A Biospecimen Collection Study to Facilitate Development of an *Ex vivo* Device Platform for Culture, Immune Assay, and Biobanking of Leukapheresis-Derived Circulating Tumor Cells, Immune Cells, and Progenitor Cells

PROTOCOL #: 0001

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without the written consent of *BioCytics, Inc.* (referred to throughout this protocol as simply *BioCytics*). These restrictions on disclosure will apply equally to all future information supplied to you which is indicated as privileged or confidential.

Sponsor Agreement and Signature Page
I have reviewed the protocol design, approve the study, and will maintain the sponsor's obligations for data monitoring, device application development, and validation.

_____ _____
Sponsor's Signature: Date
John Powderly MD, CPI
Chief Executive Officer, *BioCytics, Inc.*

Principal Investigator Agreement and Signature Page
I have read the following protocol BioCytics 0001 - A Biospecimen Collection Study to Facilitate Development of an *Ex vivo* Device Platform for Culture, Immune Assay, and Biobanking of Leukapheresis-Derived Circulating Tumor Cells, Immune Cells, and Progenitor Cells.

I agree that the protocol contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the conduct of the study.

I will use only the informed consent/HIPAA authorization approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and will fulfill all responsibilities for submitting pertinent information to the IRB/IEC responsible for this study.

I agree that *BioCytics, Inc.* or its representatives shall have access to any source documents from which case report form information, methods, and/or research data may have been generated.

I further agree not to originate or use the name of *BioCytics, Inc.* in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol, without the prior written consent of *BioCytics, Inc.*

_____ _____

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1. INTRODUCTION AND RATIONALE

1.1. Current Therapies Ineffective

Current standards of care for cancer treatment, such as surgical resection, chemotherapy, and radiation therapy, are often ineffective. This may be due to resistance mechanisms including DNA damage repair, multi-drug resistant (MDR) pump or P-glycoprotein expression, and alteration of drug targets.[1,2] Moreover, the genetic basis of cancer suggests that a patient-specific or individualized therapeutic approach may be more beneficial to patients than the historical "one size fits all" chemotherapy treatment regimens available today as standard of care. Specifically, targeted therapy and immunotherapy have gained popularity and shown clinical efficacy upon failure of frontline treatments.

Immunotherapy has shown promising results in the treatment of cancer with the potential for long-term survival – particularly in hypermutated cancers such as melanoma, lung cancers, and GI cancers.[3] Immunotherapy involves administration of checkpoint inhibitors, monoclonal antibodies, vaccines, and even live immune cells (cell therapy) to help the patient's immune system better act against cancer (cancer.gov). The most prevalent and clinically effective form of immunotherapy is immune checkpoint inhibition (ICI) – a type of drug that blocks immunosuppressive molecules (such as PD-1/PD-L1 and CTLA-4) that prevent immune cells from killing cancer cells (NCI). Immune checkpoint blockade of PD-1/PD-L1 has shown varied benefit across hematological and non-hematological cancer types, including a 40-45% objective response rate (ORR) in melanoma and non-small cell lung cancer (NCSCL), 13-24% overall response rate in urothelial cancer, and 87% objective response rate in relapsed/refractory Hodgkin's lymphoma.[4] However, only a fraction of patients benefit from ICI due to the complex nature of antitumor immunity. While monoclonal antibodies (mAb) may successfully block immune checkpoints, deficiencies in patients' immune systems often prevent resident lymphocytes from mounting an effective anti-tumor response. To combat this challenge, focus has widened to modifying, stimulating, and expanding immune cells *ex-vivo* for therapeutic infusion.

At present, there are nearly 400 cancer cell therapies in development in the United States alone.[5] T cell therapy is the most abundant approach to cell therapy for cancer treatment and represents the two newest market entries for cancer cell therapy (Novartis' Kymriah and Kite/Gilead's Yescarta). Adoptive cell therapy (ACT) using T cells can generally be divided into three approaches: (1) Tumor infiltrating lymphocytes (TIL), (2) cells with genetically engineered T cell receptor (TCR), and (3) chimeric antigen receptor (CAR) T cells.[6] Excluding TIL therapy, T cells are generally collected through apheresis of patient or healthy donor peripheral blood, synthetically activated, transduced with a CAR/TCR, expanded into clinically relevant numbers, and infused into the patient where response is monitored.[7]

Unfortunately, CAR T and TCR therapy have so far been limited to the treatment of hematological malignancies due to the scarcity of solid tumor-specific targets, difficulty in trafficking to and

infiltrating into the tumor, proliferating and functioning in the immunosuppressive tumor microenvironment (TME), and tumor antigen escape.[8] TIL therapy is also limited by the inaccessibility of the tumor tissue from which the effector cells are isolated, the paucity of rare T cells within the tumor, the attenuated proliferative ability of the T cells during *ex vivo* expansion, as well as the poor anti-tumor efficacy upon reinfusion.[6] Both CAR-T and TCR therapy also require complicated and costly gene modification to target the T cells against a certain antigen, such as CD19 or CD20 on B cell hematologic malignancies. Thus, these approaches are currently applicable for blood cancers like Non-Hodgkin's Lymphoma (NHL) and chronic lymphocytic leukemia (CLL).[9] CAR T cells are further limited by their restriction to extracellularly expressed epitopes, since they are MHC independent and thus cannot see intracellular proteins presented by MHC I. Conversely, TCR therapy is both aided and limited by its reliance on MHC interactions. Together, these three modes of adoptive T cell therapy have shown little clinical efficacy in treating solid tumors beyond TIL's success in melanoma.[10] Thus, a major unmet need exists as the top 10 most prevalent cancers are all of solid tumor origin (world cancer research fund).

To successfully treat solid tumors with T cell therapy, a personalized approach must be taken that targets multiple patient-specific neoantigens, maximizes on-target toxicity and minimizes off-tumor toxicity, and minimizes potential for graft vs host disease (GVHD) and graft rejection. In a series of seminal studies, Dr. Steven A. Rosenberg of NCI's Surgery Branch and pioneer of TIL therapy, identified the immune checkpoint programmed cell death protein 1 (PD-1) as a T cell marker of tumor specificity.[11–13] Together, these publications of the Rosenberg Lab showed that the peripheral blood and tumor infiltrating $CD8^+$ $PD\text{-}1^+$ cells possess the greatest incidence of tumor-neoantigen specificity and thus could prospectively identify the most promising lymphocyte populations for T cell therapy. In 2014, Dr. Powderly, along with collaborators from multiple institutions, published in Nature that programmed death-ligand 1 positive (PD-L1+) and cytotoxic T-lymphocyte-associated protein 4 positive (CTLA-4+) T cells in the tumor micro-environment were a predictive correlate to anti-PD-L1 antibody therapy in cancer patients.[14] These data suggest that PD-1+, PD-L1+ and/or CTLA-4+ T cells may be the most important subpopulations for future adoptive cell therapies. Further, TCR-mediated activation of T cells results in PD-1 expression where this immune checkpoint can act as a measure of peripheral tolerance. Upon resolution of the insult and decrease in antigen load, PD-1 expression falls to normal levels. However, during sustained antigen stimulation such as that seen in chronic infection and cancer, PD-1 expression on T cells remains high.[15] Thus, PD-1 may serve as a surrogate biomarker of antigen specificity.

Immune suppression, induced by self-tolerant or self-regulating immune cells, continues to be the most undesired mechanism in the effective treatment against solid tumors. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expands during cancer, inflammation, and infection, causing a remarkable ability to suppress T-cell responses. MDSCs remain the main immunosuppressive cells present in the tumor microenvironment[16] and their role in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA) as a potential

treatment option is supported due to their accumulation in sites of inflammation[17]. T regulatory cells (Tregs) also have the explicit ability to suppress T-cell activation in solid tumors.

Multiple disease models have a dysregulation of T regulatory cells (Tregs) which have a fundamental role in the maintenance of self-tolerance and immune homeostasis.[18] This myriad of autoimmune diseases and inflammatory disorders caused by a dysregulation of Tregs could benefit from further advances in their ex-vivo selection, activation, and expansion. A prominent example is Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative disease affecting both upper and lower motor neurons, causing a rapid decline of muscular control and function, leading to paralysis and eventually death. Etiology is poorly understood, and most theories focus on a complex interaction between genetic and environmental factors. Neuroinflammation, however, is the most apparent pathological signature of ALS as many patients have dysfunctional and reduced numbers of Tregs, which tend to inversely correlate with disease severity and progression.[19] As a result, during 2016 to 2018, investigators across several institutions published preliminary Phase 1 data detailing the generation of clinically relevant numbers of Tregs for the treatment of ALS. [18,20,21] Thanks to these findings, there are currently three NIH-listed clinical trials for infusion of autologous Tregs for ALS therapy (ClinicalTrials.gov).

1.2. Genomic and Proteomic Technologies

Genomic and proteomic technologies now allow physicians and scientists to focus their efforts on discovery of specific biomarkers for each patient's unique tumor mutations and neoantigens.[22] This targeted drug approach is tailored to the genomic signature (a snapshot of the genetic makeup of a tumor) of each individual tumor and is known as "Personalized Medicine." These types of studies require access to individual patient biospecimens, particularly blood and tumor tissue, in what is called a biobank or biorepository.

1.3. Circulating Tumor Cells

Menarini-Silicon Biosystems/Immunicon/Veridex LLC. (Huntingdon Valley, PA) developed a platform technology called *Cell Tracks® Autoprep System and Analyzer II* for isolation and quantitation of rare cells. Using an antibody-ferrofluid conjugate, circulating tumor cells (CTCs) can be isolated from whole blood by immunomagnetic capture. This technique has been FDA approved as an *in-vitro* diagnostic test for circulating tumor cell enumeration with indications in metastatic breast, prostate, and colorectal cancer. Such monitoring has been shown to be predictive of progression-free and overall survival in metastatic breast cancer patients.[23]

The clinical use of *Cell Tracks® Autoprep* for *in-vitro* diagnostic purposes has been exclusively licensed to Menarini-Silicon Biosystems/Immunicon/Veridex LLC. under the brand name *CellSearch™*. The reagents and cartridges for in-vitro diagnostic testing (*CellSearch™ Circulating Tumor Cell Kit*) are FDA approved for the intended use below:

The *CellSearch™ Circulating Tumor Cell Kit* is intended for the enumeration of circulating tumor cells (CTC) of epithelial origin, defined as CD45-EpCAM+Cytokeratins 8+/18+/19+ in whole blood.

The presence of CTCs in the peripheral blood, as detected by the *CellSearch™ Circulating Tumor Cell Kit*, is associated with decreased progression free survival and decreased overall survival in patients treated for metastatic breast cancer. A CTC count of 5 or more per 7.5mLs of blood is predictive of shorter progression free survival and overall survival.[23]

Over the past 10 years, multiple breast cancer clinical trials in the adjuvant Stage II and III setting, and meta-analysis have shown CTC enumeration >1/7.5mL of whole blood as level 1 evidence of a prognostic biomarker for local relapse, distant relapse and overall survival.[24,25] Therefore, monitoring remission by CTC enumeration in the adjuvant setting is becoming a shifting standard of care, especially in breast cancer and prostate cancer.[26,27]

Due to their heterogeneity and low frequency in peripheral blood, alternative methods for CTC enrichment and detection have come to the forefront of cancer research. Diagnostic leukapheresis (DLA), represents one strategy to enable more sensitive detection of rare CTCs. DLA is another innovative and shifting standard of care that enables the early detection of CTCs in patients of any stage with minimal residual disease (MRD) before metastatic progression and accompanying symptom onset. Multiple recent trials from American and European consortiums have shown that DLA for CTCs is feasible, safe, and can increase the collection of CTCs by 200-fold compared to a single 7.5 mL tube of whole peripheral blood.[28,29] Due to the potential impact of DLA for CTC enrichment to enable highly sensitive monitoring of remission status, "standardized reporting" of CTC enumeration by DLA is being adopted.[29–31]

A futuristic application of DLA is to utilize CTCs not just for early diagnosis of relapse, but also as the antigen source for therapeutic *ex-vivo* antigen presentation in an "*ex-vivo* lymph node" to develop cellular immunotherapies as outlined in the 2012 Frontiers in Oncology review article "Circulating Tumor Cells: The Substrate of Personalized Medicine".[32] This protocol will enable the collection of CTCs by DLA for both monitoring of potential relapse and to serve as the tumor antigen source for an *in vitro* generated
autologous T cell therapy to treat solid tumors.

In addition to *the* CellSearch™ Circulating Tumor Cell Kit, Menarini-Silicon Biosystems, LLC also offers the CellSearch™ Profile Kit. This kit contains the necessary reagents for CTC isolation but does not incorporate the counterstains and fixation procedures needed for enumeration of the CTC population. The CellSearch™ Profile Kit is intended to be used for live CTC isolation and downstream applications such as RNA isolation for microarray analysis and even culture and expansion.

1.4. Immune Cells

We plan to isolate PD-1$^+$ and/or CTLA4$^+$ lymphocytes from study subjects through peripheral blood or leukapheresis by utilizing conjugated antibodies. These are fully human antibodies that have been FDA approved for clinical trials, with known safety and toxicity data. Antibodies will be conjugated with biotin and further linked with anti-biotin microbeads or other approved methods to select the PD-1 $^+$ and CTLA-4$^+$ cells. The anti-tumor capability of those peripheral blood-derived lymphocytes will be examined. Furthermore, we will expand the PD-1$^+$ and/or CTLA-4$^+$ T cells with standard *ex-vivo* rapid expansion protocol using approved antibodies, agonists, and cytokines. We envision the future of cellular immunotherapy will be autologous peripheral blood-selected lymphocytes with the most anti-tumor specificity (i.e., PD1+ and/or CTLA4+ T cells). Furthermore, additional leukapheresis-derived cell populations will be investigated, such as monocyte derived dendritic cells (Mo-DC), naïve T cells (T_n), γδ T cells, and natural killer (NK) cells. Specifically, attempts to recapitulate antigen presentation using autologous tumor source (such as CTCs) by modeling an "*Ex vivo* Lymph Node" will be further investigated.

Similar methodologies may be applied for the isolation of immune suppressive cells, such as Tregs and MDSCs, obtained via peripheral whole blood and possibly leukapheresis from patients with a history of autoimmune disease. These cells may go through further selection, enrichment, and activation in culture to acquire proficiency in these expansion and enrichment assays intended to further cell therapy advances for autoimmune disease and inflammatory disorders.

1.5. Hematopoietic Stem and Progenitor Cells

The implications of translational research regarding the use of hematopoietic stem and progenitor cells (HSPCs) are vast. The engineering of HSPCs to induce pluripotent stem cells (iPSCs) for the development of organoids and subsequent differentiation into many different tissue types, may have revolutionary implications for regenerative medicine, drug discovery and personalized medicine (Harvard Stem Cell Institute). CD34+ and CD133+ are cell surface markers that have been shown to express themselves in many hematopoietic stem and progenitor cells, with the latter as a transmembrane glycoprotein expressed in normal and cancer stem cells. We plan to isolate CD34+ and CD133+ from peripheral whole blood and leukapheresis PBMCs of healthy donors and cancer patients without the use of mobilizing agents, to further our preliminary translational efforts in cancer immunotherapy, as well as for biobanking and non-clinical Research Use Only (RUO) sourcing to potential external collaborators and vendors.

1.6. Cell Culture Technologies

Immortalized cancer cell lines are frequently used in biomedical research to evaluate the toxicity and efficacy of potential therapies. While useful, these lines are often artificially transformed and may develop significant genetic drift from the original sample.[34] Patient-derived primary tumor

cells provide the ideal model system when studying cancer *ex-vivo*. However, primary cancer cells are difficult to isolate, expand *in-vitro*, and display heterogeneity that typed lines do not. Additionally, biopsy sampling is highly invasive to patients and many tumor locations are inaccessible by needle. Alternative sources for obtaining primary tumor cells include enrichment from peripheral blood (CTCs), ascites fluid (breast, GI, ovarian, and others), pleural effusion (lymphoma, breast, lung, ovarian), and DLA material (cancer.net). Together, these methods are either palliative or noninvasive compared to biopsy sampling and will undoubtedly be powerful tools for developing personalized cancer medicine.
This research protocol will investigate the isolation of circulating tumor cells and immune cells that can be propagated in culture for viability and apoptosis assays and/or cryopreservation.

In this protocol, described in more detail below, subjects who are eligible and consent to BioCytics 0001 and who are deemed clinically eligible for leukapheresis per the Principal Investigator or treating oncologist may undergo the leukapheresis procedure, in which approximately 80-200mL of a mononuclear cell product will be collected with Terumo BCT's Spectra Optia® Apheresis System utilizing their Continuous Mononuclear Cell Collection (CMNC) program or Mononuclear Cell Collection (MNC) program. Cells of interest will be isolated from the apheresis product using immunomagnetic selection on the CliniMACs Prodigy® or other approved methods. Isolated CTCs, immune cells and progenitor cells will be expanded in culture using routine aseptic technique and cell culture methodology. Tumor cells expanded in culture will be cryopreserved and used to develop viability and potency assays. Cells may be banked for the duration of the study, and then may be discarded or archived as anonymous tissue, or they may be sourced to external collaborators or vendors for nonclinical RUO. Immune cells of interest will then be selected based on their potency against tumor cells during co-culture assays, or due to their function of interest, such as in the treatment of autoimmune diseases.

Biospecimen collection of peripheral whole blood, fluids and tissue from consented subjects may undergo the same selection, expansion, and culture methodologies as the methods used for leukapheresis cellular products. As such, CTCs, immune cells, mesenchymal stromal cells, and hematopoietic stem and progenitor cells will be isolated and expanded in culture using sterile technique and cell culturing methodologies. These cells expanded in culture will be cryopreserved and used to develop viability/apoptosis assays. T-Lymphocytes will then be selected based on their efficacy to eliminate tumor specific CTCs.

1.7. Tumor Resistance and Sensitivity Assays

There are two main types of tumor assays to predict responsiveness to immunotherapy and/or chemotherapy: growth-based tumor resistance assays and death-based tumor sensitivity assays.

Growth-based assays measure a fraction of tumor cells surviving exposure to an immunotherapeutic and/or chemotherapeutic agent. Cell growth-based assays detect drug resistance of tumor cells.

Death-based assays measure a fraction of tumor cells killed by exposure to a chemotherapeutic and/or immunotherapeutic agent. Cell death-based assays detect drug sensitivity of tumor cells.

Chemotherapy is known to kill tumor cells by two major mechanisms: necrosis and apoptosis. High doses of chemotherapy induce necrotic cell death while milder, repeated therapeutic doses induce apoptotic death. Modern targeted drugs engage apoptotic pathways to induce cell death.

Induction of apoptosis has been shown to correlate with response and survival in human cancers.[35,36] Cell growth-based drug resistance assays do not measure apoptosis and, thus, cannot be used to directly determine drug sensitivity of tumor cells. Therefore, the older tumor resistance-sensitivity assays may be useful for chemotherapy choices but are not predictive for modern targeted drug mechanisms.

1.8. Growth-Based Tumor Resistance Assays

Growth-based tumor resistance assays use a patient's viable tumor cells harvested from a fresh biopsy or surgical resection, paracentesis, thoracentesis, leukapheresis or peripheral whole blood to determine tumor resistance to different autologous T lymphocytes. An example is listed below:

1.8.1. Mitochondrial Tetrazol Assay (MTT)

The MTT Assay is a rapid viability assay that measures the enzymatic reduction of a tetrazolium salt by tumor cells surviving drug exposure. The reduction of MTT to the purple formazen product only occurs in viable cells.[37] The colorimetric absorbance can be measured between 500 and 600 nm by a spectrophotometer.

The MTT assay has several disadvantages including: 1) not all cell types metabolize MTT and 2) production of the formazen product is dependent on the MTT concentration in the medium and time. Thus, the kinetics of the reaction must be determined for each cell type analyzed. This can be laborious and is unsuitable for the personalized medicine approach.

1.9. Tumor Apoptosis-Viability Assays (TAVA)

Modern laboratory techniques can accurately measure *in vitro* tumor apoptosis-viability assays (TAVA) using colorimetric, fluorescent, or luminescent labeling techniques. These methods have even become automated with flow cytometers and digital imaging software that determine stoichiometric apoptosis/viability ratios with specificity and sensitivity down to a single cell. Since tumor cells are either viable (alive) or apoptotic (impending death), TAVA methods are more precise than older proliferation-based assays. Historical growth-based assays may have indeterminate results from a low proliferation index (if cells do not grow).

Modern TAVA does not depend upon cell growth and may be analyzed immediately on circulating tumor cells without incubation or growth. Certain tumors that are slow growing (such as breast or prostate cancer) may be ideal for TAVA. Furthermore, TAVA may be less error prone than growth-based lab culture techniques and thus be more reproducible as a standardized assay.

Currently, there is no FDA approved *in vitro* diagnostic kit for tumor resistance-sensitivity or apoptosis-viability assays, nor has there been validation of older resistance proliferation methods to test modern TAVA. As such, *ex vivo* validation, *in vivo* clinical response correlation, and device kit development may enable rapid *in vitro* diagnostic TAVA reports from circulating tumor cells. This futuristic kit would bypass the need for invasive surgical biopsy of fresh tumor cells and thus enable real-time (without incubation or cell growth) targeted drug analysis of personalized medicine through serial blood draws.

1.10. Apoptosis Pathways and Development of Tumor Apoptosis-Viability Assays (TAVA)

Recent studies have dissected the molecular events associated with activation of apoptotic pathways. Activation and execution of these apoptotic pathways requires a family of proteases known as caspases.[38] These pathways can be divided into 2 components: the intrinsic pathway that involves release of factors from mitochondria and the extrinsic pathway that is engaged after death receptor-ligand binding.[39] The intrinsic pathway involves release of cytochrome c and other factors from mitochondria, formation of the apoptosome, and consequential activation of caspase 9. In the extrinsic pathway, death receptor and ligand association lead to Fas Associated Death Domain Protein (FADD) aggregation and caspase 8 recruitment and activation. Both the intrinsic and extrinsic pathways converge on activation of downstream caspases including caspase 3 to initiate the morphological features associated with apoptosis.[39] In addition, the two apoptotic pathways are linked by the cleavage of the pro-apoptotic protein BID via caspase 8 and its subsequent translocation to the mitochondria.[39]

Induction of apoptosis in a cell population is often a heterogeneous event in that not all cells initiate and complete apoptosis simultaneously. Thus, when performing apoptosis assays on treated cells *ex vivo*, it is beneficial to assay multiple time-points. Alternatively, to ensure that an apoptotic event is detected, one may use assays that incorporate multiple apoptotic markers that detect apoptosis at various stages.

Historical apoptosis assays used for research purposes include:

a) Microculture Kinetic (MiCK) Assay for Apoptosis at *Diatech Oncology, LLC* (McGill University, Montreal QC Canada)
b) Adenosine Tri-phosphate Tumor Chemosensitivity Assay (ATP-TCA) at *Laboratory for Applied Neoplasia Research and Cytostatics Evaluation, Inc.* (LANCE) (Bonn, Germany)
c) Fluorescent Nuclear Stain Viability Assay, ChemoFx at *Precision Therapeutics, Inc.* (*Pittsburgh, PA*)

d) Chemosensitivity TCR Assay at *Oncovation Laboratories, Inc.* (New York, NY)

The above assays detect one endpoint to measure apoptosis. Our future goal is to develop a TAVA that incorporates multiple apoptotic markers. This may include Annexin V staining, measuring caspase activity, and/or M30 antibody staining [recognizes the CK18-Asp^{396} neo-epitope (M30) of caspase-cleaved cytokeratin 18 (CK18); the M30 antibody is a well-established apoptotic marker that does not stain viable or necrotic cells].

1.11 Anti-tumor Lymphocyte Cytotoxicity Assay (Cytotoxicity Assay)

Functional tolerance may further be investigated by autologous tumor cell cytotoxicity assays. Functional video microscopy, such as Essen Bioscience's IncuCyte Zoom® Live Cell Analysis System may be used in these assays.

1.12 COVID-19 Research

The rationale for amending the protocol to expand the aim of immunology research into COVID-19 was made due to the clinical and scientific expertise of the Principal Investigator and his scientific team, the study site's apheresis equipment and clinical lab infrastructure, and lastly *BioCytics'* research lab infrastructure; all of which could be directly applied to viral immunology research, potentially benefitting the COVID-19 pandemic crisis. The expansion aim was first drafted to include healthy adult volunteers convalescing from COVID-19 to acquire PBMCs and plasma from these individuals to aid in the discovery of a vaccine or monoclonal antibody treatment against SARS-CoV-2 (the pandemic viral pathogen responsible for COVID-19 disease). This amendment was made possible via the Letter of Amendment (LoA) version 1-Apr-2020, IRB approved on 24-Apr-2020. The second revision for COVID-19 research occurred through a revised LoA version 16-Sep-2020, IRB approved 09-Oct-2020. The second revision was amended to primarily to incorporate SARS-CoV-2 diagnostic testing via the collection of biospecimen samples from nasal, nasopharyngeal (NP) and oropharyngeal (OP) swabs and microcapillary sampling in both children (5-17 years of age) and adults with suspected or confirmed COVID-19 with the purpose of supporting EUA device kit or test validations.

To create an ease of interpretation at the study site(s), the two IRB approved Letter of Amendments for COVID-19 research, version 1-Apr-2020 and version 16-Sep-2020 respectively, will be fully incorporated into this protocol as pertinent. Thus, this protocol amendment version 09-Dec-2020, will supersede LoA version 16-Sep-2020, enabling all aspects of this research study to be carried out under this protocol.

The aim of COVID-19 research in this protocol is intended to be applicable to other viral pathogens capable of pandemics and not limited to SARS-CoV-2. However, currently the protocol is meant specifically for COVID-19 related research and any change will require further clarification and/or amendment and submission to the IRB for approval.

2. STUDY DESIGN

This is an observational prospective laboratory biospecimen collection and device/platform development protocol to collect data for future device/platform submission for "device/platform approval" by the FDA. There may be other applications that are discovered with the use of this device or through biospecimen collection. This study also serves as the means to function as a biobank to support future scientific investigation across a multitude of fields outside of cancer immunotherapy, such as regenerative medicine, cell therapy development for autoimmune and inflammatory disorders, and for vaccine or therapeutic discovery for infectious viral pathogens of pandemic nature.

2.1. Objectives

2.1.1 Primary Objective

This is a study to investigate the feasibility of harvesting, expanding, and selecting T lymphocytes from cancer patients and healthy volunteers. The preliminary objective of this study is aimed at selecting PD-1^{+} and CTLA4^{+} T cells and other cellular fractions from peripheral blood of cancer patients and healthy volunteers by using specific conjugated antibodies, evaluating their functional *ex vivo* anti-tumor cytotoxicity against targeted autologous tumor cells.

2.1.2 Secondary Objectives

- Develop methodology and proprietary techniques towards collecting and expanding viable circulating tumor cells and immune cells collected through leukapheresis/apheresis.
- Develop methodology and proprietary techniques towards using viable circulating tumor cells and immune cells for tumor apoptosis-viability assay (TAVA) after exposure to chemotherapy and immunotherapy targeted drugs *ex vivo*, and lymphocyte tumor cytotoxicity assays.
- Optimize rapid expansion protocol for PD-1+ T cells, and other leukapheresis derived cell fractions.
- Upon validation of BioCytics circulating tumor cell TAVA, and lymphocyte tumor cytotoxicity assay, an FDA Pre-market Notification 510(k) application for *in vitro* device platform will be submitted.
- An FDA Investigational New Drug (IND) application for Lymphocyte T cell immunotherapy platform will be submitted.
- Continued assessment of bioinformatics, cell processing, genomic, and cryogenic infrastructure needed to create a biobank for tumor cells, immune cells, and hematopoietic stem and progenitor cells.
- Banking and sourcing of plasma, tumor cells, and immune cells from peripheral blood for facilitation of biomarker discovery.

- Develop methodology for isolation, selection, enrichment, and expansion of immune suppressor cell fractions, such as Tregs and MDSCs, for the advancement of therapeutic cell therapy of autoimmune and inflammatory disorders.
- Develop preliminary methodology for isolation, selection and cryopreservation of hematopoietic stem and progenitor cells from apheresis without mobilization.
- PBMC and plasma isolation from whole blood and apheresis of convalesced COVID-19 subjects for biobanking.

2.2 Endpoints

2.2.1 Primary Endpoint

Successful selection of PD-1^+ and CTLA4^+ T cell, and other immune cellular fractions from peripheral blood or leukapheresis of study subjects by using antibody conjugation, and evaluation of functional *ex vivo* anti-tumor cytotoxicity against targeted autologous tumor cells.

2.2.2 Secondary Endpoint

Prove hypothesis that selected and expanded PD-1^+ and CTLA-4^+ T cells, and/or other cellular fractions, have higher anti-tumor cytotoxicity than un-selected T cells from either patient peripheral whole blood or leukapheresis product.

Use this proof of principal, to then optimize a model platform for *ex vivo* T cell expansion towards an FDA IND submission for human applications on a clinical trial, *BioCytics*' Protocol #0002.

2.3. Selection of Study Population

Study participants include adult cancer patients of all stages of solid tumor origin and healthy volunteers. Healthy volunteers may vary in nature and can be further classified based on different criteria, as best summarized in section 2.3.1. below and section *10.7 Appendix 7.* Healthy volunteers' scope of participation is primarily due to whether the healthy volunteer is deemed by the Principal Investigator or treating physician as "truly healthy" with no clinically significant medical disease or disorder, or if the healthy volunteer is "otherwise healthy". The latter is due to the subject having a history of autoimmune disease, inflammatory disorder, or is suspected/diagnosed with COVID-19 disease yet is otherwise clinically stable.

Adult healthy volunteers that fall into this second category will be carefully assessed by the Principal Investigator or treating physician to assure subject safety and data integrity. Subject eligibility criteria will also be carefully assessed and organized accordingly as to what it is pertinent and/or necessary to fulfill. With respect to COVID-19 and its disease transmission, healthy volunteers may also include pediatric subjects, aged 5-17 if parental consent is granted and assent where applicable is obtained. Lastly, healthy volunteers may also include consented employees from either *BioCytics'* or the study site(s)'; however, most healthy volunteers are community-based individuals and not employees. Consented employees may mostly participate in whole blood collection for instrument or device validation purposes, yet due to the COVID-19

pandemic, employees who are suspected of or have been diagnosed with COVID-19 may wish to participate in collection of biospecimens for that aspect of this study.

2.3.1. Study Population & Sample Size

The number of study subjects to be enrolled in this prospective observational biospecimen collection laboratory research study is approximately 1,500. This target population may be increased if needed; however, proper notification and approval by the IRB/IEC (Institutional Review Board/Independent Ethics Committee) must occur.

The population of patients to be enrolled for this study will consist of two cohorts: 1) cancer patients, and to a lesser extent 2) healthy volunteers.

1) **Cancer patients** must have a solid tumor histological diagnosis and can be at any stage of disease progression, including in the pre-surgical/neoadjuvant setting, receiving adjuvant therapy, and those considered in remission. Most cancer patients are referred to the study site(s) by their treating oncologists; however, a portion are self-referrals.

   Cancer patients receiving any form of treatment, including those participating in clinical trials with experimental study drugs, will be allowed to participate in this observational biospecimen laboratory study. While enrollment will be geared towards patients with histologically proven metastatic solid tumors, a portion of the subjects may be non-metastatic solid tumor cancer patients in the neo-adjuvant, adjuvant, and in remission settings.

   Cancer patients in the earlier stages of disease progression serve to research the baseline status of a patient's immune system in their tumor prior to receiving any treatment, to attempt to culture tumor infiltrating lymphocyte (TILs), and lastly, to attempt to "filter-out" or collect CTCs downstream of the leukapheresis procedure or upon peripheral whole blood collection. As referenced in section 1.3. of this protocol, collection/filtration and enumeration of CTCs may serve as a diagnostic companion and promising prognostic tool in the adjuvant setting.

2) **Healthy volunteers** make up the second, smaller study population for this observational biospecimen laboratory study. Healthy volunteers serve primarily to aid in the proficiency, quality control and/or optimization of study procedures, experimental design assay development, and for device/equipment validation. Most healthy volunteer adult subjects are referred to the study site(s) by community-based word of mouth, however a smaller portion of them may be employees of either the study site(s) or of *BioCytics.*

   The classification criteria seen below, serve to aid study design and to help identify the component(s) a study subject may participate in at a given time point based on their eligibility criteria and the cohort they are in.

Classification Criteria for Healthy Volunteer Cohort:

- Subjects with no pre-existing medical conditions or disorders, deemed as "truly healthy" by the Principal Investigator or treating physician's discretion.
- Subjects with a history of autoimmune diseases and/or inflammatory disorders who are otherwise considered "healthy" by the Principal Investigator or treating physician's discretion.
- Subjects who are employees of the study site(s) and/or *BioCytics*
- Subjects who have been diagnosed with presumable or confirmed viral pandemic pathogens, including but not limited to SARS-CoV-2, either in the acute, sub-acute, and convalesced state, but who are otherwise considered "healthy" by the Principal Investigator or treating physician's discretion.
    - Children from 5-17 years of age may participate in this study based on specific minimally invasive biospecimen collection procedures (outlined below and in section *3.2.1. Procedures by Study Visit*) and with appropriate parental consent, and child assent where possible.

Due to the COVID-19 pandemic and the consequent amendment to aid in the research response, study subjects with COVID-19 disease may participate in all aspects of this protocol, but depending on their disease state, infection timeline, and age, participation may be limited to only one component, procedure, and/or type of biospecimen collection. Age is a very important criteria, as pediatric subjects (5-17 years old) will only be eligible to participate in minimally invasive biospecimen collection procedures. These procedures will be limited to the collection of swabs (nasal, nasopharyngeal and/or oropharyngeal), microcapillary samples, and saliva or urine collection.

Healthy volunteers, including those with history of autoimmune diseases or inflammatory disorders may participate in collection of biospecimen samples, primarily through peripheral whole blood draws or leukapheresis, since these may be beneficial for future cell therapeutic indications utilizing enrichment and expansion assays of Tregs and MDSCs. Healthy volunteers who have no history of disease or medical condition may participate in leukapheresis for preliminary attempts at isolation, selection and cryopreservation assay development of hematopoietic stem and progenitor cells (HSPCs) without the use of mobilizing agents. Upon optimization of these techniques, a protocol specific for the mobilization of HSPCs to the peripheral circulation in healthy volunteers must be drafted and submitted for approval, as mobilizing a patient would require a separate clinical trial protocol.

2.3.2. Study Duration

The open enrollment period for the study is anticipated to be for at least 5 years from the time of approval or until 1500 study subjects have been enrolled. Once enrolled, study subjects will remain on the study to be followed for biospecimen collection. Study subjects will be followed until the study closes or a patient is discontinued due to screen-failure, withdrawal, or disease progression and death.

It is anticipated that cancer patients may remain on study throughout the natural history and progression of their disease. The study duration may overlap with multiple lines of anti-cancer therapy, and as long as patient performance status remains ECOG $\leq$3 for patients not undergoing leukapheresis and ECOG ≤1 for patients undergoing leukapheresis collection. Cancer patients' tumor status and survival data may be followed through communication with the clinic site for another 5 years after active enrollment has stopped.

Healthy volunteers and surviving cancer patients (those most likely in earlier stages of disease progression or considered in remission), will remain on the study until the study closes or the subject discontinues or withdraws consent. Healthy volunteers will not be followed up annually unless clinically indicated by the Principal Investigator or treating physician. Additionally, COVID-19 healthy volunteers may be discontinued prior to study closure if BioCytics 0003, a protocol specific for pandemic viral pathogens including SARS-CoV-2, is drafted and submitted for approval. In the event that IRB approval is obtained, adults with COVID-19 may be asked to either continue their participation in BioCytics 0001 as a healthy volunteer for other aspects of this protocol or be removed from the study altogether. These adult subjects may then be asked to continue their COVID-19 research participation via the new study protocol BioCytics 0003. However, all pediatric patients enrolled in BioCytics 0001 for COVID-19 research would be discontinued from this study upon implementation of protocol 0003. This is due to BioCytics 0001 having no indication for pediatric healthy volunteers outside the scope of COVID-19 research.

2.3.3. Inclusion Criteria

Informed consent must be obtained prior to any research procedures related to this protocol taking place and must follow ICH-GCP guidelines. After study subjects sign the Informed Consent Form they will be evaluated for study eligibility. Subjects who meet the inclusion criteria and who do not meet any of the exclusion criteria will be eligible for study participation.
Due to the COVID-19 pandemic and the nature of disease transmission, the informed consent process may occur remotely in healthy volunteers participating in COVID-19 research. The remote consent process for COVID-19 study subjects has been previously approved by the IRB and must occur following the study site(s)' remote consent policies and procedures. This includes parental consent of minors 5-17 years of age, where if applicable and technologically possible, child assent should be obtained following the same remote procedure.

In the event regulatory authorities, accreditation agencies, or external collaborators or vendors require additional testing for blood borne communicable or infectious diseases based on

emerging epidemiological risks or intended use of the biospecimen product, this study will be amended accordingly and re-submitted for IRB for review.

Inclusion criteria will be itemized based on what cohort the study subject is in due to the wide range of study subjects able to participate in this observational biospecimen collection study.

Cancer Cohort Inclusion Criteria:

A subject will be eligible for study participation if he/she meets the following criteria:

1. Male or Female Adult ≥ 18 years of age.
2. Histological diagnosis of any solid tumor type and at any stage of disease progression including in the neoadjuvant/presurgical setting, adjuvant setting, or considered in remission.
3. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 to 3 (see Appendix 2) and an estimated life expectancy of at least 3 months.
4. Subject or subject's legal representative provides written informed consent.
5. Negative serology screening test for HIV, Hepatitis B surface antigen, and Hepatitis C antibody, or negative reflex PCR test result for HIV, Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV)
    a. If serologies are pending, and biospecimen(s) have been collected, they may be quarantined per the study site(s)' policies and procedures until serology comes back negative unless the biospecimen product has been shipped to an external collaborator or vendor, in which case the product will be labeled appropriately, and the client notified of the results upon becoming available.
    b. Viral serologies may be repeated throughout the course of this study independently of a negative test result.
6. Additional eligibility criteria need to be met for leukapheresis collection:
    a. ECOG Performance Status must be 0 or 1 (see Appendix 2)
    b. Screening laboratory values must be collected and resulted on the day of leukapheresis collection, prior to the procedure being performed:
        - WBC ≥2000/µL
        - Neutrophils ≥1000/µL
        - Platelets ≥100x10^3/µL
        - Hemoglobin ≥9 g/dL
        - Creatinine ≤2.5 x ULN
        - AST ≤2.5 x ULN without, and ≤ 5 x ULN with hepatic metastases
        - Bilirubin ≤2 x ULN (except patients with Gilbert's syndrome, who must have total bilirubin ≤ 3.0 mg/dL)
        - Negative urine pregnancy test for women of childbearing potential

Healthy Volunteer Cohort Inclusion Criteria:

A subject will be eligible for study participation if he/she meets the following criteria:

1. Male or Female Adult ≥ 18 years of age.

   a. Pediatric healthy volunteers from 5-17 years of age, with suspected or confirmed COVID-19 diagnosis by laboratory test will be eligible to participate in minimally invasive biospecimen collection procedures, as long as written parental consent has been obtained, and if applicable and technologically able, child assent. Minimally invasive biospecimen collection procedures allowed for pediatric participation include swabs (nasal, NP and OP), microcapillary sampling, and saliva or urine collection.

2. Healthy volunteers are eligible, including the following:
   a. History of autoimmune disease or inflammatory disorder considered clinically stable by the Principal Investigator or treating physician's discretion.
   b. Suspected or diagnosed COVID-19 disease by laboratory test, whether in the acute, sub-acute or convalesced state.
   c. Employees of the study site(s) or *BioCytics*, as long as fulfilment of inclusion criteria 3.c is obtained (see below).
3. Subject or subject's legal representative provides written informed consent.
   a. For pediatric healthy volunteers, written informed consent from parent or legal guardian. If the child or adolescent, is able to provide written informed consent, assent is to be obtained in addition to parental consent were applicable.
   b. For healthy volunteers with suspected or confirmed COVID-19 disease, including pediatric subjects from 5 – 17 years of age, written informed consent may be obtained remotely utilizing a secure electronic signature platform, such as DocuSign.
   c. If the subject is a current employee of the study site(s) or *Biocytics*, the latest version of the IRB approved employee subject material titled *Employee Consent Note for Clinical Trial* must be obtained in addition to the main ICF.
4. Negative serology screening test for HIV, Hepatitis B surface antigen, and Hepatitis C antibody, or negative reflex PCR test result for HIV, HBV, and HCV.
   a. If serologies are pending, and biospecimen(s) have been collected, they may be quarantined per the study site(s)' policies and procedures until serology comes back negative unless the biospecimen product has been shipped to an external collaborator or vendor, in which case the product will be labeled appropriately, and the client notified of the results upon becoming available.
   b. For acute and convalescing COVID-19 healthy volunteers, viral serology may not be performed at enrollment & screening due to the infectious nature of SARS-CoV-2. However, negative test results for viral serologies must be obtained prior to leukapheresis collection (refer to inclusion criteria 5.b.i. and 5.b.ii.).
   c. If the subject is a current employee of the study site(s) or *Biocytics* and participation is limited to biospecimen collection (not leukapheresis collection), viral serologies will not be performed in order to abide by PHI/HIPPA law. However, if the employee decides to participate in leukapheresis collection, viral serology and safety labs must be performed (refer to inclusion criteria 5.c. and 5.d.).

5. Additional eligibility criteria need to be met for leukapheresis collection:
    a. Male or female adult ≥ 18 years old.
    b. For convalesced COVID-19 healthy volunteers:
        i. Negative PCR for SARS-CoV-2 and symptom resolution for ≥ 28 days from symptom onset in healthy volunteers previously diagnosed with COVID-19 by laboratory test.
        ii. Negative serology test for HIV, Hepatitis B surface antigen, and Hepatitis C antibody, or negative reflex PCR test result for HIV, HBV and HCV.
    c. For employees of the study site(s) or *BioCytics*, negative serology test for HIV, Hepatitis B surface antigen, and Hepatitis C antibody, or negative reflex PCR test result for HIV, HBV and HCV must be obtained.
    d. For all healthy volunteers, screening laboratory values must be collected and resulted on the day of leukapheresis collection, prior to the procedure being performed:
        - WBC ≥2000/µL
        - Neutrophils ≥1000/µL
        - Platelets ≥100x10^3/µL
        - Hemoglobin ≥9 g/dL
        - Creatinine ≤2.5 x ULN
        - AST ≤2.5 x ULN
        - Bilirubin ≤2 x ULN (except patients with Gilbert's syndrome, who must have total bilirubin ≤ 3.0 mg/dL)
        - Negative urine pregnancy test for women of childbearing potential

2.3.4. Exclusion Criteria

Subjects must not meet any of the following exclusion criteria to be eligible to participate in this biospecimen collection study.

1. Subjects with active infection requiring therapy (fever, localizing source) will be excluded until the infection resolves.
    a. This excludes subjects with suspected or confirmed COVID-19 by laboratory test while in the acute and sub-acute phase of viremia.
2. Underlying medical condition that, in the Principal Investigator's or treating oncologist's opinion, will obscure the interpretation of the patient's safety.
3. Confirmed positive reflex PCR test result for HIV, Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV).

3. STUDY METHODS

3.1 Assignment (Enrollment) to Study

Study participation begins once written informed consent is obtained by a study team member, following ICH-GCP guidelines. Study subjects, whether healthy volunteers or cancer patients, will

be entered into *BioCytics'* database, generating a BioCytics 0001 study subject enrollment ID number, and pertinent screening evaluations may begin to assess study eligibility (inclusion/exclusion) criteria. This enrollment number will be used to identify the patient throughout the entire study participation.

Subjects who do not meet the additional eligibility criteria at a given time point for leukapheresis collection will not be considered a screen fail for any aspect of this study if at a later date their additional eligibility criteria results are met. Data collected on screening failures will not be entered in the clinical database, and once assigned, subject numbers for any screening failures, discontinued, or withdrawn cancer patients and/or healthy volunteers will not be re-used.

3.2. Clinical Procedures

3.2.1. Procedures by Study Visit

The study is divided into two types of visits: Screening Visit (after informed consent has been obtained) and Study Visits (for *Time and Events Schedule*, see Appendix 1). Since this is an observational prospective biospecimen collection laboratory study, there is not a defined screening window needed for a subject to be enrolled. Both subject cohorts (cancer patients and healthy volunteers) are pre-identified as potential candidates by the Principal Investigator or treating oncologist's discretion for either biospecimen collection, leukapheresis collection, or both. However, in order to participate in leukapheresis collection, a subject must meet the additional leukapheresis eligibility criteria (see section *2.3.3 Inclusion Criteria*) and express a willingness to participate in this aspect of the study. These criteria must be met on day the of leukapheresis collection prior to the procedure from taking place.

Adult healthy volunteers with suspected or confirmed COVID-19 disease may participate in either biospecimen or leukapheresis collection; however due to the nature of disease transmission, while not fully convalesced their participation will be limited to biospecimen collection only. In addition, screening viral serology for HIV, HBV and HCV will not be collected in these adult subjects while in the acute and or sub-acute stage of disease since the collected biospecimen(s) would be considered and handled as a category B substance regardless of the serology results. However, prior to leukapheresis collection, a convalesced subject must have negative viral serology results for HIV, HBV and HCV (or negative reflex PCR) and a negative SARS-CoV-2 PCR test result.

In these COVID-19 convalescing healthy volunteers, biospecimen(s) collection(s) may occur remotely or in a designated area outside the study site(s). Biospecimen collections, particularly nasal, nasopharyngeal (NP), or oropharyngeal (OP) swabs may be obtained by self-administration, or if remotely, by a contracted and licensed healthcare professional, such as a phlebotomist, medical laboratory technician (MLT), registered nurse (RN), nurse practitioner (NP), physician assistant (PAC) or medical doctor (MD). Important to note, in pediatric COVID-19 subjects, biospecimen collection is limited to minimally invasive procedures obtained in the

presence of a parent or legal guardian. These include nasal, NP, and/or OP swabs, saliva and/or urine collections, and microcapillary sampling. Additionally, no viral serology will be obtained in these subjects as they require a peripheral whole blood draw, which is outside the scope of pediatric participation.

Screening Visit

The screening visit may occur at any time and regardless of other concomitant treatment regimens.

Cancer Cohort Screening Visit Includes:

- Introduction of BioCytics 0001
- Eligibility review
- Informed consent and signature
- Enrollment and assignment to subject ID number
- Screening baseline reference labs: CBC, CMP, urine pregnancy test (if woman of childbearing potential), and viral serology for HIV, hepatitis B surface antigen, and hepatitis C positive RNA with reflex PCR
- ECOG Performance Status determination
- Prior and concomitant anti-cancer treatment documentation
- Tumor pathology report documentation
- Tumor Status (NED, PD, SD, PR, CR) as determined by the treating oncologist
- Tumor markers may be drawn as deemed appropriate by treating oncologist (i.e., CEA, CA19-9, PSA, CA27-29, CA-15-3, CA 125)
- If applicable, additional biospecimen collection may be obtained at screening (i.e., peripheral whole blood, microcapillary sampling, discarded fluid, saliva, urine, and/or stool collection)
- Possible 5-20 minutes of light to moderate exercise prior to whole blood draw(s)

Healthy Volunteer Cohort Screening Visit Includes:

- Introduction of BioCytics 0001
- Eligibility review
- Informed consent and signature
- Enrollment and assignment to subject ID number
- Viral serology for HIV, hepatitis B surface antigen, and hepatitis C positive RNA with reflex PCR for all adult healthy volunteers, excluding convalescing COVID-19 healthy volunteers and healthy volunteers who are current employees of *BioCytics'* and/or the study site(s)' who choose not to participate in leukapheresis collection
- Screening baseline reference labs of CBC, CMP, and urine pregnancy test (if woman of childbearing potential) are only required for adult healthy volunteers who are potential

leukapheresis candidates or who have a history of autoimmune disease or inflammatory disorder
- If applicable, additional biospecimen collection may be obtained at screening (i.e., peripheral blood, microcapillary sampling, nasal, NP and OP swabbing, saliva, urine, and stool collection)
- Possible 5-20 minutes of light to moderate exercise prior to whole blood draw(s)
- For pediatric subjects (5-17 years of age), biospecimen(s) collection(s) limited to minimally invasive procedures: swabbing (nasal, NP and/or OP), microcapillary sampling, saliva and/or urine collection

Study Visit(s):
For the cancer cohort, each study visit will mostly occur at a patient's next scheduled treatment or follow-up visit; however, study visits may be scheduled specifically for biospecimen collection or leukapheresis collection that fall outside of their routine scheduled visits. Leukapheresis collection visits will occur no more frequently than every 4 weeks, and will not occur while a patient is actively on an investigational therapeutic clinical trial. Biospecimen collection visits may occur more frequently as long as deemed appropriate by the principal investigator or treating oncologist's discretion.

For the healthy volunteer cohort, visits may occur no more frequently than every 4 weeks for leukapheresis collection. Biospecimen collection visits may occur more frequently as long as deemed appropriate by the principal investigator or treating physician's discretion.

Cancer Cohort Study Visit(s) Includes:
- ECOG Performance Status determination
- Adjuvant treatment documentation
- Tumor status (NED, PD, SD, PR, CR) as deemed necessary by the treating oncologist
- Tumor markers may be drawn as deemed appropriate by treating oncologist (i.e., CEA, CA19-9, PSA, CA27-29, CA-15-3, CA 125)
- If applicable, additional biospecimen collection may be obtained at screening (i.e., peripheral whole blood, microcapillary sampling, discarded fluid, saliva, urine, and/or stool collection)
- Possible 5-20 minutes of light to moderate exercise prior to peripheral whole blood draw or leukapheresis collection
- If leukapheresis collection visit, CBC, CMP, and urine pregnancy test (if women of child-bearing potential), will be performed prior to the leukapheresis procedure taking place, as an additional safety measure.

Healthy Volunteer Cohort Study Visit(s) Includes:
- Treatment documentation if healthy volunteer has a clinically significant condition, such as an autoimmune disease or inflammatory disorder

- Additional biospecimen(s) collection(s) including peripheral whole blood, microcapillary sampling, saliva, urine, and/or stool collection, and nasal, NP and/or OP swabbing (if COVID-19 subject)
- Possible 5-20 minutes of light to moderate exercise prior to peripheral whole blood draw or leukapheresis collection
- If leukapheresis collection visit, CBC, CMP, and urine pregnancy test (if women of child-bearing potential), will be performed prior to the leukapheresis procedure taking place.

For both cohorts, additional biospecimen collection(s) and/or additional donor required labs and questionnaires may occur that are currently not delineated here if future research design or external collaborations and/or biospecimen vendors require them based on the intent of the use of the biospecimen collected, i.e. biospecimen product intended for clinical use in an allogeneic recipient. If this becomes pertinent, this protocol will be revised and submitted for approval prior to implementation of changes or an entire new protocol may be submitted for revision and approval.

The frequency of biospecimen collections may vary between study visits; however for the purpose of this study, whole blood collection (via central port or peripheral venipuncture) will be limited to no more than approximately 100mLs at any one study visit or no more than approximately 550mLs in an 8-week period. This is the case for both cohorts as long as safety labs are within delineated levels and deemed safe by the Principal Investigator or treating oncologist's discretion, particularly for cancer patients (see section *2.3.3. Inclusion Criteria* and *3.2.3 Safety Parameters*).

Microcapillary blood draws, also known as capillary blood sampling, may occur more frequently since these avoid the effects of blood volume reduction and reduce significantly the risk of anemia due to excessive phlebotomy.[40] Healthy Volunteers may be asked to donate blood for research purposes to be used as quality control or optimization of procedures and processes.

For cancer patients and healthy volunteers who consent to leukapheresis collection, study collection visits will occur no more frequently than every 4 weeks. Healthy volunteers may be asked to perform leukapheresis for proficiency, quality control, and optimization of research procedures. Cancer patients may participate in leukapheresis collection if they are not actively enrolled in an investigational therapeutic clinical trial; however, they may participate if they are in-between clinical trials.

3.2.2. Mobilizing Immune Cells with Exercise

Among some of the most reproduced findings in exercise physiology is the biphasic transient lymphocytosis that is evidenced in the peripheral circulation in response to a 45 to 60 minute acute aerobic exercise bout.[41] In fact, Simpson et al. have reported that a single bout of dynamic exercise has been shown to elicit a profound and almost instantaneous mobilization of all major

leukocyte subtypes into peripheral circulation.[42,43] This phenomenon, known as "exercise-induced leukocytosis", is thought to be a consequence of demargination of leukocytes from the vascular, pulmonary, hepatic and splenic reservoirs to the main blood flow of the peripheral circulation. The increases in cardiac output, blood pressure, and blood flow is accompanied by activation of the sympathetic nervous system and in turn aids in the hemodynamic shear stress causing the adherent leukocyte subtypes to detach from their endothelial ligands and enter the circulation.[43]

Important to note is that this leukocytosis is not uniform in phenotype nor function. Since the mobilization of certain types of lymphocytes is also dependent on catecholamines like epinephrine and norepinephrine which bind to the β-adrenergic receptors expressed on the lymphocytes that express these receptors in greater amount, these are subsequently mobilized in greater amount to the peripheral circulation[41–43]. Lymphocyte subtypes that are more cytotoxic in nature, particularity NK cells, γδ T cells and memory-effector CD8+ T cells preferentially express these β-adrenergic receptors on their cell surface and in the presence of catecholamines, are mobilized into the circulation with exercise.[43,44]

Due to numerous publications that have demonstrated that acute dynamic exercise mobilizes immune cells into the main axial blood flow, promoting exercise as an economic adjuvant may improve cell collection for therapeutic applications in cell therapy.[44] Since these cytotoxic cellular "fractions" or subtypes are among interest for this pilot study, study subjects may be asked to participate in light to moderate exercise (e.g., 5 to 20 minutes of jumping jacks or cycling on a stationary bike) in an effort to mobilize their lymphocytes just prior to leukapheresis collection appointments, as well as peripheral whole blood draws, for downstream culture and/or other applications.

3.2.3. Safety Parameters

Standard clinical procedures of aseptic technique for collecting blood from a patient's vein or central access port will be strictly followed. Only qualified clinical staff will perform clinical procedures of accessing central ports or phlebotomy. Measures will be taken to ensure the procedure is as comfortable as possible. Additional safety precautions following the CDC's Corona Virus 2019 Biosafety Guidelines should be implemented by all clinical staff involved in drawing suspected and confirmed SARS-CoV-2 patient blood specimen(s), and *BioCytics'* staff involved in handling, processing, and packaging the COVID-19 blood sample(s) to outside collaborators or partners. Any processing of COVID-19 suspected or confirmed blood or other biospecimen(s) should be done under a Class II Biological Safety Cabinet (BSC) and measures should be implemented to provide an additional barrier between specimen and personnel. When these COVID-19 patient specimen(s) are packed and shipped, guidelines emitted by the most current edition of IATA should be followed to ensure samples are shipped out with the appropriate UN 3373 Biological Substance, Category B label.

Patients on other clinical trials with experimental study drugs may be allowed to participate in this observational biospecimen collection laboratory study if allowed by the other trial. Monitoring patients for any ongoing symptomatic anemia (such as moderate fatigue, and/or orthostatic dizziness), and monitoring Hgb/Hct values provide additional safety measures to prevent excessive phlebotomy in both cancer and healthy volunteer cohorts. For cancer patients, additional discretion will be implemented by *BioCytics*' study team to minimize whole blood draw volume needed for experimental research and biobanking requirements. If deemed safe by the Principal Investigator or treating oncologist's discretion, these patients will have no more than approximately 100mLs of whole blood drawn at any one study visit or no more than approximately 550mLs drawn in an 8-week period. For the healthy volunteer cohort, as long as the subject is otherwise healthy and hemodynamically stable in the treating physician's discretion, there may be up to approximately 100mLs drawn per week. IRB approval must be obtained prior to any exception to the 550 mL limitation in an 8-week period (NIH Guidelines – Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center, June 2009).

Leukapheresis collection should not occur while the patient is actively dosing on an investigational therapeutic clinical trial. However, for the cancer cohort, study subjects may participate in leukapheresis collection when they are "washing out" from their last dose of an investigational therapeutic trial, and before beginning a new investigational therapeutic clinical trial. In addition, leukapheresis collection will not be performed more frequently than every 4 weeks.

3.2.4. Tumor Status

Tumor status, as defined by the Response Evaluation Criteria in Solid Tumors (RECIST), will be documented by the treating oncologist.

Tumor status may be classified as: No Evidence of Disease (NED), Progressive Disease (PD), Stable Disease (SD), Partial Response (PR), or Complete Response (CR). For adjuvant stage I, II, & III cancer patients in "remission", prognostic risk factors that predict relapse may also be tracked (based on their stage prognosis). Tumor status will be obtained from the treating oncologist, if deemed appropriate, at each study visit.

Tumor markers (CEA, CA19-9, PSA, CA27-29, CA15-3, CA-125, and/or circulating tumor DNA (ctDNA) or urinary tumor DNA (utDNA) may be drawn at baseline and monthly, according to standard of care and as deemed appropriate/necessary by the treating oncologist.

Tumor status and tumor markers will assist in correlation of viability-sensitivity assays, and correlation of circulating tumor cell enumeration with clinical response.

3.2.5. ECOG Performance Status

The Eastern Cooperative Oncology Group Performance Status (ECOG PS) will be collected at the screening visit as a baseline for all study subjects; during subsequent study visit(s), ECOG may be

determined by the treating oncologist and/or Principal Investigator's discretion. Only an ECOG ≤ 1 will be considered acceptable for potential leukapheresis collection, whereas for peripheral whole blood collection, capillary blood sampling, discarded tissue and/or fluid collection, as well as other biospecimen collection (i.e., saliva, urine and/or stool), an ECOG ≤3 will be deemed acceptable.

3.2.6. Concomitant Anticancer Therapy Documentation

Concomitant chemotherapy, biological therapy, hormonal therapy, immunotherapy, or investigational therapy will be allowed during this trial as deemed appropriate standard of care by the treating oncologist. The prior and concomitant anti-cancer therapy regimen will be recorded as part of the study data collection as documented in the Electronic Medical Records (EMR) system at each visit.

3.2.7. Blood Draw for Circulating Tumor Cells and Immune Cells

Using aseptic technique, no more than approximately 100mLs of whole blood from the patient's vein or central access port will be collected for the purpose of this biospecimen collection study during an individual study visit, with no more than approximately 550mLs drawn over an 8-week period. Specialized tubes for maintaining viability of tumor cells and immune cells will be supplied to the site(s) and utilized when deemed appropriate by the principal investigator and/or treating oncologist. In addition, capillary blood draw(s) may occur as needed by experimental design and/or collaborations. Peripheral whole blood draws may be paired with other biospecimen samples, such as tumor tissue, saliva, urine, and stool.

3.2.8. Biopsies

For the purpose of this study, tumor tissue may be collected in cancer patients either through core needle biopsies or surgical resection of the tumor. Core needle biopsies may be performed solely for research purposes by an interventional radiologist under imaging guidance and collected only if there is an acceptably low risk of mortality/serious morbidity. For patients with newly discovered cancers in the pre-surgical or neoadjuvant setting, *BioCytics* may coordinate with the patient's surgeon at the time of their standard medically necessary tumor surgical resection in order to obtain a baseline status of the patient's immune system in their tumor microenvironment, as well as downstream culture applications for both tumor cells and immune cells such as tumor infiltrating lymphocytes (TILs), in addition to furthering genomic and proteomic technologies and antitumor lymphocyte cytotoxicity assays.

3.2.9. Leukapheresis Collection for Circulating Tumor Cells, Immune Cells and Progenitor Cells

The intent of leukapheresis in both cancer patients and adult healthy volunteers for the purpose of this study is vast (as referenced in sections 1.3, 1.4, 1.5, and 1.12). Among the most noteworthy is the collection of CTCs including CD133+ cells, collection of immune cell fractions, possible collection of hematopoietic stem and progenitor cells without mobilization, and lastly collection of PBMCs and plasma for prophylactic and therapeutic development against SARS-CoV-2. As a result, cancer patients who are not actively participating in therapeutic experimental clinical trials

and adult healthy volunteers who are eligible for leukapheresis collection, study collection visits for leukapheresis will occur no more frequently than every 4 weeks. Leukapheresis will be performed on the Terumo BCT Spectra Optia® or other FDA approved apheresis devices. The collection will be performed based on the study site(s)' approved leukapheresis SOPs when clinical and laboratory safety parameters are met on collection day.

The collection duration may vary depending on the subject cohort and intent of collection; however, most collections will take approximately 1.5 to 2 hours, as long as the safety of the patient is maintained, with an ideal collection time of 1 total blood volume (TBV) processed. Continuous mononuclear cell collection (CMNC) or mononuclear collection (MNC) program will be utilized on the Terumo BCT Spectra Optia® device so that mostly white blood cells are removed, with just a small number of platelets and red blood cells found in the final collection product. The rest of the cells and plasma in most instances will be returned to the patient. However, plasma may be collected in addition to MNC collection during the CMNC procedure if experimental design or biospecimen sourcing require additional plasma. In such cases, a small volume of approximately 20-300mLs of plasma may be collected during a single leukapheresis CMNC collection procedure. The allowed maximum of 15% of TBV of plasma may be collected at a given timepoint, which in an average adult constitutes a collection volume of approximately 700mLs. Therefore, no more than 15% of TBV may be collected during one leukapheresis procedure, occurring no more than once in a 4-week time period. Plasma may be collected for prophylactic and therapeutic monoclonal antibody discovery/development against SARS-CoV-2, or to bring the PBMC volume in a leukapheresis product to its desired cellular concentration.

At this time, *BioCytics'* use of biospecimens for its internal research efforts or as a Biobank, is strictly for non-clinical RUO (Research Use Only) and not for clinical or allogeneic recipient use. Therefore, the recommendations from the FDA regarding blood banking establishments and required donor screening labs for transmissible blood borne pathogens, HLA matching, ABO typing, and donor history questionnaires and accompanying materials (refer to Appendix 10.6) will not be utilized at the study site(s) and has been removed from other aspects of this protocol. In the event that the intent of the biobank, and subsequent use of blood and tissue products being sourced, is changed to incorporate clinical/cGMP/recipient use, this protocol will be amended and submitted for IRB review, abiding by FDA guidance for all donor related necessary procedures to comply with clinical grade cGMP products/use.

3.2.10. Microaggregate Filter for CTC Collection During Leukapheresis

Modern transfusion medicine and hemoperfusion practices utilize filtration methods to remove unwanted cells or microaggregate debris. Examples include the historical use of leukocyte depletion filters (LDFs) and modified leukocyte depletion filters (mLDFs) used for blood transfusions to remove donor WBCs to prevent recipient alloreactivity or transfusion reactions. Another example is micro-aggregate filtration during intra-operative autologous blood salvage to remove harmful blood component microaggregates (e.g., fibrin clots) and non-blood component particulate matter (e.g., fat emboli).

It was historically reported that that the use of autologous non-filtered intra-operative salvaged blood may contribute to the risk of recurrence, mortality and shorter survival time in patients with malignancies.[45] However, recent publications have been able to demonstrate the effectiveness of utilizing microaggregate filters for the removal of tumor cells during cell salvage intra-operatively in onco-surgery.[45,46]

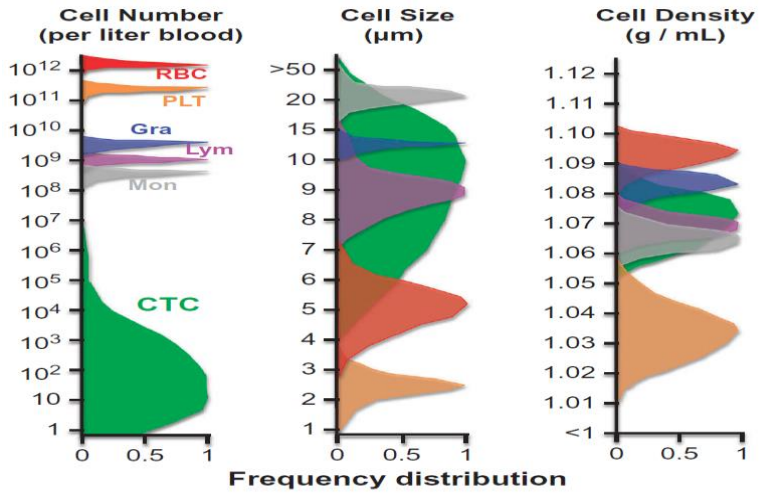
Additional studies have demonstrated that these microaggregate LDF and mLDF blood filters have been successful in minimizing adverse effects of autologous banked blood use in onco-surgery.[45,47] They have been studied, designed and utilized based on the FDA approved predicate models such as: Heamonetics® SQ40™ (a microaggregate blood transfusion filter for intraoperative blood salvage) and Leukogard LG-6, Pall© (a leukocyte reduction arterial blood filter). The latter was the first clinical attempt utilizing a leukocyte depletion filter during oncologic surgery that successfully demonstrated patients tolerating the application of the system without problems during operative and postoperative follow-up.[48,49]

As is well known, circulating tumor cells (CTCs) are generated when individual, or groups of cells, detach from the solid tumor, whether primary or metastatic, and enter the peripheral circulation. Although CTC's are rare in peripheral blood, their presence indicates a systemic disease stage and detection of CTC's in peripheral blood have been useful in estimating prognosis and disease progression in breast, prostate, skin and colon malignancies.[50] Due to the clear prognostic and diagnostic value of CTCs for cancer patients of all stages, *BioCytics* hypothesizes that they may be depleted with 40µm microaggregate blood filters such as those utilized in intraoperative blood salvage to remove harmful microaggregates and non-blood particulate matter.[51] By connecting the filters during the leukapheresis procedure where approximately 1 total blood volume is circulated through the Terumo BCT Spectra Optia® apheresis CMNC procedure, there is an increased possibility of isolating CTCs or CTC clusters within the microaggregate blood filters. Therefore, this study proposes to incorporate the use of an FDA approved 40µm microaggregate blood filter (e.g., Heamonetics® SQ40™ microaggregate high blood flow filter) to be connected downstream of the leukapheresis procedure for CTC isolation.

The use of the aforementioned SQ40™ µm filter, or other similar FDA approved filters ranging from 40µm, provide a very low residual volume which minimizes red blood cell loss and its high filtering flow rate capacity allows a reduction in the number of administration sets.[51,52] Thus, this may allow the filter to be placed in series or in parallel, utilizing a range of approximately 1 to 4 filters per leukapheresis collection procedure. *BioCytics* proposes that leukapheresis filtration of harmful microaggregates such as CTCs and CTC clusters, may allow further research into potential benefit by calculating pre & post leukapheresis CTC reductions from cancer patients' whole blood. In addition, the placement of these FDA approved microaggregate blood filter(s) will not adulterate the CMNC program nor IDL kit of the Terumo BCT Spectra Optia® apheresis machine as these will be placed under aseptic technique on the return IV of the cancer patient and follow *BioCytics* strict SOPs. Our goal is to obtain the most leukocytes from the counter-centrifugation cell density layering by the Spectra Optia device into the leukapheresis product; and to

simultaneously obtain the most CTCs from the 40 micron in-line filter at the return IV of the patient. We also plan to compare pre & post CTC counts before and after leukapheresis to determine the CTCs in the patient, leukapheresis product, and on the filter.

Based on 40 micron screen filter size, leukopenia is not anticipated post leukapheresis collection with the utilization of microaggregate blood filter(s) in the return IV of the patient.[53] Lymphocytes range from 8-12µm, while CTC's range from 15-40µm and CTC clusters range from 40-100 µm. Lastly, *BioCytics* internal SOP requires that all patients undergoing leukapheresis collection be closely monitored by the Principal Investigator and/or treating oncologist, including complete blood counts pre and post leukapheresis collection. To date, of the 106 successful unfiltered leukapheresis collection procedures done at Carolina BioOncology Institute, there have not been any cases of a clinically significant reduction in leukocytes post leukapheresis collection among all patient cohorts, furthering *BioCytics* belief that the use of these FDA approved in-line microaggregate blood filters will not cause clinically significant leukopenia. Reference diagram below.[54]



3.2.11. Long Term Follow-Up Period

Upon completion of enrollment/screening visits and study visits, the patient will have completed the active phase of the protocol. Thereafter, the patient will enter a long term follow up period whereby survival data, tumor status, and concomitant adjuvant therapy in cancer patients may be followed through communication with the study center for an additional 5 years, as deemed appropriate by the Principal Investigator. Healthy volunteers will not be followed long-term upon study completion unless deemed appropriate by the Principal Investigator or treating physician.

3.2.12. Study Completion, Follow-up, and Close Out

Upon completion of enrollment visits for approximately 1500 patients enrolled in this observational prospective biospecimen collection laboratory study, the study will close to accrual and all active patient study visits will end, unless the study population is augmented per the

Principal Investigator's discretion and formal approval of the study-specific IRB is obtained. Upon completion of the 5 year follow up period for all 1500 patients, the study will be completed and undergo formal close out.

3.2.13. Subject Withdrawal

Patients and/or healthy volunteers may be removed from study for any of the following reasons and at any time point:

- Patient choice to withdrawal consent
- Patient decline in performance status: ECOG ≥ 3
- Patient transition to home hospice and/or be unable or unwilling to return to clinic due to tumor progression
- Patient death
- At the discretion of the treating oncologist or Principal Investigator that the patient's condition or quality of life is adversely affected by repeated phlebotomy or other study procedures related to this study
- The scope of a study amendment no longer includes participation of study subject, (i.e., pediatric COVID-19 subjects)

3.2.14 Possible Toxicities

Possible toxicities or side-effects, as they relate to this observational biospecimen collection study will depend on the procedure(s) taking place. Among the most common are side-effects associated with blood draws, apheresis and biopsies/tumor resection. These are itemized below and must be disclosed by the study staff at the time of the informed consent procedure.

Possible side-effects or toxicities related to monthly blood draws may include:

- Symptomatic anemia from phlebotomy blood loss (rare)
- Discomfort at the site of blood draw such as redness, bruise, swelling (common)
- Venipuncture risk of superficial thrombophlebitis (rare)
- Port infection if drawn from central line access (rare)

Side effects from apheresis were reported in two large studies totaling 47,856 subjects.[55,56] No life threatening, serious, or long-term problems were reported. Possible, although rare, risks and discomforts include the following:

<u>Mild (Occurred in less than 1.3% of procedures):</u>

- Access problems
- Hypotension
- Tingling
- Device Problems
- Urticaria
- Nausea/Vomiting
- Hematoma at puncture site

- Hypertension
- Flush
- Phlebitis
- Shivering, fever
- Arrhythmia
- Back Pain
- Vertigo

Severe (Rare, occurred in less than 0.11% of procedures):

- Hypotension, syncope
- Urticaria
- Fever, chills
- Nausea, vomiting
- Access Problem
- Flush
- Tingling, stitching,
- Arrhythmia

Extremely rare events (occurred in less than 0.01% of procedures):

- Bronchospasm
- Technical problem, wrong plasma
- Abdominal pain, gastrointestinal bleeding
- Back pain, spasm
- Epilepsy
- Hypertension, asystole, chest pain
- Anaphylaxis, adverse event to drug
- Anxiety, hyperventilation

Common risks which eligible leukapheresis subjects should be informed of include the following:

- Most people feel tired. Patients should plan on little or no activity for the next 12 hours.
- Patients might have dizziness, fainting, and nausea. The risk of dizziness or fainting was lower during leukapheresis than in many studies of whole blood donation.
- Leukapheresis may temporarily lower patient platelet count. Therefore, patients should avoid activities that would put them at risk for bruising or bleeding.
- Patients may bruise more easily during the next couple of days.
- Leukapheresis may temporarily lower patient white blood cell count. Therefore, patient(s) should notify their doctor if they develop any signs of infection such as a fever.
- Patients might develop redness or pain where the needle was. They should be instructed to call the study doctor/study staff if this happens.
- Patients should be instructed to drink plenty of fluids.
- If patients get dizzy, they should be instructed to lie down with their feet elevated above their head if possible.

- For the next 5 hours, patient(s) should leave their bandages on and keep them dry. Patients should not do any heavy lifting or exercise during this time.

The risk of a biopsy is dependent upon where the tumor is located in a patient's body and the type of procedure it is, core needle biopsies versus surgical resection of a tumor. It is possible that the research biopsy or procedures may involve risks to patients that are not currently known or foreseeable. The treating oncologist or principal investigator will discuss additional personal risk as it relates to a biopsy procedure or surgical resection in detail. Patients may need to sign additional consent documents required by the local hospital where the biopsy or surgery is taking place.

Risks of core needle biopsies include the following:

Common - Expected to occur in 10%-25% of people:

- pain from the procedure
- bruising and soreness at the site of biopsy
- scarring at the site of biopsy
- radiation risks as mentioned above if the biopsy is done in combination with a CT scan

Rare - Expected to occur in less than 1% of people:

- infection
- internal bleeding

3.3. Laboratory Procedures

3.3.1. Processing and Isolation of Tumor, Circulating Tumor Cells, Immune Cells and Progenitor Cells

Within 24-72 hours after blood draw or leukapheresis collection, the PBMC product obtained from peripheral whole blood and/or leukapheresis will be processed according to *BioCytics'* internal SOPs. Tumor cells, immune cells, and progenitor cells may be immuno-magnetically captured or flow sorted, also in accordance with *BioCytics'* SOPs. The isolated tumor cells (whether obtained from liquid biopsy measures or discarded tissue), immune cells, and progenitor cells may then be cultured, harvested, or cryopreserved. These cells may be stored in BioCytics' biobank or biorepository and sourced for research use only to external collaborators and/or vendors for research across all scientific fields and not limited to immunotherapy cancer research.

3.3.2. Harvesting of Circulating Tumor Cells, Immune Cells and Progenitor Cells

Using standard cell culture techniques, tumor cells, immune cells and progenitor cells may be expanded according to *BioCytics*' internal SOPs.

3.3.3. Cryopreservation of Circulating Tumor Cells, Immune Cells and Progenitor Cells

Using standard cryopreservation techniques, tumor cells, immune cells and progenitor cells may be rate-controlled frozen, after appropriate selection, flow sorting or alternative approach, and possible expansion, enrichment and harvesting, in accordance to *BioCytics'* internal SOPs.

3.3.4. Storage of Plasma for Biomarker Discovery

Using standard centrifugation techniques, plasma may also be separated from whole blood or collected from the leukapheresis CMNC procedure and frozen according to *BioCytics'* internal SOPs for future biomarker discovery and biobanking/biorepository storage and sourcing.

4. ADVERSE EVENT REPORTING

Since this is an observational prospective biospecimen collection laboratory study, adverse events are not anticipated. Although not anticipated, any adverse event (AE) or serious adverse event (SAE) whether attributed or related to study procedures occurring in a patient after providing informed consent, will be documented in the study site(s)' electronic medical records (EMR), as well as tracked in *BioCytics'* data logs. AEs and SAEs are reviewed by the Principal Investigator, as well as by the Quality Systems Manager and QA study team members during the monthly QA meetings.

All SAEs and AEs will be assessed by the Principal Investigator or treating oncologist for severity, relationship to the investigational/study procedure(s) and whether the event meets criteria of an SAE. If criteria are met for a serious adverse event, whether attributable or related to study procedures, the SAE will be reported by the Principal Investigator to *BioCytics* immediately, and no later than 24 hours of learning of its occurrence. *BioCytics* may also contact the Principal Investigator to obtain further information on a reported Serious Adverse Event. *BioCytics* may also report the SAE to its respective Institutional Review Board immediately, and no later than 24 hours of learning of its occurrence and document all said communication/correspondence between all parties: investigator, sponsor, and IRB/IEC.

To date, *BioCytics* has observed one unexpected and unrelated death the day after a blood donation. Upon the Principal Investigator's knowledge of this event, the IRB was immediately notified, and documentation of this event was entered in the site's EMR. With respect to leukapheresis, to date there have been 135 leukapheresis collection procedures, out of which there have been only 14 mild reactions reported, constituting 10.37% of the total runs. These AE's were assessed by the clinical staff and most were due to transient mild symptoms of citrate related hypocalcemia, manifested as a mild peripheral sensory paresthesia (tingling) in toes and peri-orally, which resolved by giving an additional oral calcium carbonate (Tums) and reducing the pheresis flow rate. Other mild AE symptoms related to leukapheresis were mild chills (or

feeling cold) which resolved with a warming blanket and feeling symptoms of hunger (mild hypoglycemia) which resolved with a sports drink. Lastly, there have not been any clinically significant venipuncture related issues observed in the 587 patients that have been enrolled to date.

5. STATISTICAL METHODS

5.1. Sample Size Determination

An approximate of 1500 cancer patients and/or healthy volunteers are to be enrolled in this study. The sample size is not determined from a power analysis and may be incremented based on the Principal Investigator's discretion and upon IRB/IEC approval.

5.2. Statistical Consideration

The study is not powered for prospective statistical significance, however retrospective statistical tests may be applied to determine preliminary trends between tumor viability/apoptosis assays (TAVA), lymphocyte tumor cytotoxicity assay (LTCA), CTC enumeration, phenotyping, lymphocyte flow cytometry, and clinical tumor responses.

5.3. Statistical Analysis

All data will be listed individually by study subject. For quantitative parameters, summaries for descriptive statistics will include the mean, standard deviation, minimum, median, and maximum. For qualitative parameters, summaries for descriptive statistics will include the frequency and percentage.

6. VALIDATION OF TUMOR APOPTOSIS-VIABILITY ASSAY (TAVA) AND LYMPHOCYTE TUMOR CYTOTOXICITY ASSAYS (LTCA)

Validation of *BioCytics'* TAVA and (LTCA) will be compared to other tumor profiling assays that may have already been performed as clinical standard of care (such as Foundation One, Caris, ImmunoSeq).

Apoptosis is characterized by loss of membrane integrity and the appearance of "membrane blebs" due to the ballooning appearance of the cell membrane. These morphological changes can be monitored using time-lapse video microscopy. As an alternative validation approach, we will confirm our TAVA & LTCA results by examining morphological features of apoptosis.

Results from the TAVA & LTCA may be reported to the treating oncologist for research purposes only. These research results will not affect or change standard of care or treating regimens established by the National Comprehensive Cancer Network (NCCN) guidelines.

7. ETHICAL ASPECTS

7.1. Compliance

7.1.1. Good Clinical Practice

This study must be carried out in compliance with the protocol and in accordance with *BioCytics'* internal SOP. These are designed to ensure adherence to Good Clinical Practice, as described in the following documents:

i) ICH (International Conference for Harmonization) Harmonized Tripartite Guidelines for Good Clinical Practice 1996; E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018
ii) Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community
iii) Declaration of Helsinki, concerning medical research in humans

The Investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice to which it conforms. A copy of the Declaration of Helsinki is located on the internet at: http://www.wma.net.

7.1.2. Good Laboratory Practices (GLP) and Good Tissue Practices (GTP)

The trial will follow GLP and GTP requirements (see 21 Code of Federal Regulations Part 58 (GLP) and Part 1271 (GTP). Efforts are currently being made for the adherence of Good Manufacturing Processes (GMP) for future IND submission of immune-cell therapy autologous investigational product, as well as for future biobanking and sourcing of cells or cell products for clinical use.

7.2. Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed Informed Consent Form/HIPAA authorization, and other information to study subjects must be reviewed by an Independent Institutional Review Board/Independent Ethics Committee (IRB/IEC) per GCP guidelines. A signed and dated statement that the protocol and informed consent form have been approved by the IRB/IEC must be given to *BioCytics* before study initiation. The IRB/IEC will be notified for any and all amendments to this protocol, including administrative changes and those that change the study design. These changes in turn need approval by the IRB/IEC prior to implementation at the study site(s). Annual review of this protocol will be submitted to the IRB for continued approval or at a frequency deemed necessary by the IRB, regardless of a Change in Research (CIR) submission. In addition, the Principal Investigator will be required to submit, maintain, and archive essential study documents according to ICH GCP guidelines.

7.3. Informed Consent

The Principal Investigator or designee (clinical research coordinator) will explain to each patient (or legally authorized representative) the nature of the research study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort which may occur. Patients will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time and that withdrawal of consent will not affect their subsequent medical treatment or relationship with the treating physician/oncologist.

Information regarding incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed because of participation in study procedures can be found in the informed consent form.

This informed consent should be given by means of a standard written statement, written in non-technical language. Only in the case that we anticipate consenting non-native English speakers, language translations of the informed consent will be submitted to the IRB/IEC for approval and become available to the patient if requested. In the case of an emergency situation the site counts with staff members fluent in Spanish, Japanese, Hindi and Arabic; however, we do not anticipate screening/enrolling patients not fluent in English. The patient should be given ample time to read over the consent form and have all of their questions/concerns answered prior to signing and dating it, along with the signature and date of the person who administered the informed consent; once voluntary written consent is given, the patient should be given a copy of the signed document.

The informed consent form is part of the protocol and must be submitted by the Principal Investigator (along with the protocol) for IRB/IEC approval. *BioCytics* supplied a proposed informed consent form, which complied with regulatory requirements is considered appropriate for the study. Any changes to the proposed consent form by the Principal Investigator must be agreed to by *BioCytics* before submission to the IRB/IEC, and a copy of the approved version must be provided to *BioCytics* after IRB/IEC approval. Changes to the consent form requested by the IRB/IEC are to be provided to *BioCytics* for review prior to implementation.

Biocytics' Principal Investigator and/or designee (research coordinator) will make sure that the most updated and current version of the Informed Consent Form (ICF) approved by the IRB/IEC will be given to the patient when obtaining informed consent.

7.4. Financial Disclosure

BioCytics, Inc. (referenced throughout this protocol as *BioCytics*) is a start-up biotech corporation that is being incubated in an oncology research clinic: Carolina BioOncology Institute, PLLC. The Principal Investigator and treating oncologist, Dr. John Powderly, is also Chief Executive Officer of *BioCytics* and thus has financial and proprietary interests in successful development of this technology.

7.5. Conflicts of Interest

Conflicts of interest include Dr. John Powderly's role as treating oncologist, Founder/Chief Executive Officer of *BioCytics, Inc.* and as Principal Investigator. This information will be fully disclosed in the informed consent form (ICF).

7.6. Protection and Monitoring of Conflicts of Interest

Patients enrolled in this research study will undergo informed consent with full written disclosure of the conflict of interest with Dr. John Powderly. The human cancer cell lines and plasma obtained from this protocol will have commercial value and may be used for commercial production, device kit purposes, in addition to research purposes. The research protocol will be submitted to and monitored by an Independent Institutional Review Board for ethical review and conflicts of interest.

8. BIOREPOSITORY FUNCTION AND GUIDELINES

The Biorepository will follow guidelines issued by the NCI for NCI-Supported Biorepositories (Appendix 3) and NCI Best Practices for Biospecimen Resources. At this time, *BioCytics'* biobank is intended to aid the advancement of its internal cellular immunotherapy device/platform efforts, as well as to source properly consented biospecimen samples from cancer patients and healthy volunteers for non-clinical RUO.

8.1. Biospecimen Processing, Storage and Retrieval

All specimens will be tracked using a computer inventory system. A unique identifier will be tied to all clinical and pathological data associated with a sample.

Cells will be stored at -80°C or below using a standard liquid nitrogen tank. Stabilizing agents (e.g., DMSO) will be used to preserve viability. Freezing and thawing procedures will be done following *BioCytics'* internal SOPs.

8.2. Quality Assurance/Quality Control

SOPs will be printed in a manual or in an electronic database and made available to the appropriate personnel. SOPs will describe all procedures in detail and allow for revisions or changes that are specifically documented. The document control database system, which houses all SOPs and controlled document versions at the study site(s), is called Title 21 Health Solutions.

All equipment will have regular preventive maintenance scheduled. In addition, emergency power systems, equipment monitoring, and alarm systems will be in place in case of power failures or an emergency.

8.3. Biosafety

Universal precautions and personal protective equipment will be used when handling biospecimens. Personnel exposures will be documented, and treatment protocols will be prepared in the event of a potential exposure. All biospecimen collections should be quarantined per *BioCytics*' SOPs until viral testing for HIV, Hepatitis B and Hepatitis C for the respective study subject are resulted, unless otherwise specified (refer to section 2.3.3. and 3.2.1.).

As a result of COVID-19 research, additional safety precautions should be made at the study site(s) following the CDC's Corona Virus 2019 Biosafety Guidelines. Processing of COVID-19 suspected or confirmed blood or other biospecimen(s) should be done under a Class II Biological Safety Cabinet (BSC) and measures should be implemented to provide an additional barrier between specimen and personnel. When these COVID-19 patient specimen(s) are packaged and shipped, guidelines emitted by the most current edition of IATA should be followed to ensure samples are shipped out with the appropriate UN 3373 Biological Substance, Category B label.

8.4. Biorepository Informatics

Clinical information and medical care about each biospecimen will be linked to a study number/product number. The study and/or product number will be used to track processing, storage, and distribution of the sample. The informatics system will allow for linking of biospecimens with associated research data, clinical data and results, as well as consent status.

8.5. Privacy Protection and Security

Security measures will be in place to ensure patient privacy and to allow access only to those individuals directly involved in the study. All subject biospecimens and data must be de-identified of any potentially identifying information (i.e., name, address, phone numbers) that could link external research collaborators or vendors to a study subject.

8.6. Custodianship and Intellectual Property

According to NCI Biorepository Guidelines, the biorepository staff members as custodians of the biospecimens are not considered inventors, under patent law, on future patent applications resulting from inventions made using materials distributed by the biorepository. Biorepositories have no inherent rights to future IP created by investigators that use specimens from the biorepository. However, novel downstream discoveries and applications of biorepository

specimens, such as specific cellular immunotherapy methodologies would be considered proprietary with inventorship & intellectual property rights for commercial purposes.

9. ADMINISTRATIVE REQUIREMENTS

9.1. Protocol Amendments

Any change or addition to this protocol requires a written protocol amendment that must be approved by *BioCytics* before implementation. Amendments significantly affecting the safety of patients, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB/IEC, prior to implementation at the study site(s). A copy of the written approval of the IRB/IEC must be given to the *BioCytics* monitor or their designee.

Examples of amendments requiring such approval include but are not limited to significant change in the study design, increase in the number of invasive procedures to which patients are exposed and addition or deletion of a test procedure for safety monitoring.

These requirements for approval should in no way prevent any immediate action from being taken by the Principal Investigator or by *BioCytics* in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the Investigator and is implemented by him/her for safety reasons, *BioCytics* should be notified and the IRB/IEC should be informed within 10 working days. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC approval, but the IRB/IEC of each center must be kept informed of such administrative changes.

9.2. Monitoring Procedures

Before study initiation, at a site initiation visit or at an Investigator's meeting, a *BioCytics* representative will review the protocol and electronic and/or paper case report form (CRF) with the Investigator and their staff. During the study, the *BioCytics* monitor or its designee will visit the site regularly to check the completeness of patient records, the accuracy of entries on the electronic (and/or paper) CRFs, the adherence to the protocol and to Good Clinical Practice, as well as to the enrollment progress.

The Principal Investigator must give the monitor access to relevant hospital or clinical records to confirm their consistency with the electronic (and/or paper CRF) entries. No information in these records about the identity of the patients will leave the study center. *BioCytics* monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of AEs and SAEs, and the recording of primary activity and safety variables. Additional checks regarding the consistency of the source data with the CRFs are performed according to the study-specific monitoring plan that may be carried out by either

a *BioCytics* research coordinator for self-monitoring or the Clinical Research QA department staff at Carolina BioOncology Institute (CBOI) for formal monitoring.

9.3. Recording of Data and Retention of Documents

Patient data collected on electronic and/or paper CRFs during the course of this study will be documented. Paper source documents maintained at *BioCytics* are the original signed ICFs and the enrollment eligibility criteria checklist forms. The latter serves as an aid for the clinical research personnel to ensure all steps are taken when determining study subject eligibility and for the Principal Investigator and/or treating oncologist to review prior to leukapheresis collection. All other CRFs are maintained in the EMR, which also functions as an EDC (electronic data capture) for clinical data extraction and is a key quality step in ensuring that all the data required by the protocol, regulatory compliance and/or safety measures/comments are addressed.[57]

This clinical database will be available to staff researchers. This will ensure that *BioCytics* will effectively optimize research for autologous cellular therapy. Laboratory research data is maintained in the electronic lab folder and its paper source is maintained within scientific lab notebooks and on the *BioCytics* shared server.

Commercial biospecimen vendors and/or collaborators outside of *BioCytics* who may use or purchase subjects' sample(s) for research may need to know more about subject health. While *BioCytics* may grant them access to subjects' health information, the researchers will not know subject name, address, phone number, or any other identifying information that would let them know who the subjects are. Subjects will also be de-identified in all study publications, presentations, and reports.

All information required by the protocol should be provided; any omissions require explanation. All CRFs should be completed and available for collection within a timely manner, so that the monitor may check the entries for completeness, accuracy, and legibility, ensure the CRF is signed by the Principal Investigator, or delegated staff such as a Sub-Investigator, and transmit the data to *BioCytics*. All entries to the CRFs must be made clearly, if on paper, copies must be in pen to ensure the legibility of self-copying or photocopied pages. Corrections will be made by placing a single horizontal line through the incorrect entry, so that the original entry can still be seen, and placing the revised entry beside it. The revised entry must be initialed and dated by the study staff making the entry, and correction fluid must not be used in order to adhere to Good Documentation Practice (GDP).

The Principal Investigator must maintain source documents, either electronic or paper for each patient in the study. All information on CRFs will be traceable to these source documents, which are generally maintained in the patient's file or electronic medical record. The source documents will contain all demographic and medical information, including laboratory data. A copy of the

signed Informed Consent Form (ICF) will also be kept and should indicate the study number and title of the study.

Essential documents, as listed below, will be retained by the Principal Investigator for as long as needed to comply with national and international regulations. *BioCytics* will notify the Investigator(s)/study site(s) when the study-related records are no longer required. The Investigator agrees to adhere to the document retention procedures by signing the protocol.

Essential documents include:

1. Signed protocol and all amendments
2. IRB/IEC approvals for the study protocol and all amendments
3. All source documents and laboratory records
4. CRF copies both paper and electronic
5. Signed informed consent forms for all patients screened
6. Any other pertinent study document

9.4. Auditing Procedures

In addition to the routine monitoring procedures, *BioCytics* or its designees may conduct self-audits of clinical research activities in accordance with internal SOP to evaluate compliance with the principles of Good Clinical Practices. *BioCytics*, its designee, a regulatory authority, and/or an IRB/IEC may wish to conduct an inspection during the study or after its completion. If an inspection is requested by a regulatory authority, the Investigator will inform *BioCytics* immediately that this request has been made. *BioCytics* monitoring and self-auditing may be performed by Carolina BioOncology Institute's clinical research QA department and/or a *BioCytics'* study team member. To date, *BioCytics* has conducted three self-audits in 2016, 2018 and January 2020, respectively. All pertinent findings and/or deviations were documented and reported to the study-specific IRB and maintained in source documents as well as on *BioCytics* shared server.

9.5. Handling of Anonymous Tumor Specimens Beyond Study Closure

As per the FDA guidance on leftover human specimens that are anonymous (Appendix 4), tissue collected as a result of this research study may be maintained cryopreserved, cultured, manipulated, and used for commercial and research purposes. Specimens that have been deidentified and sourced to outside collaborators or vendors may be subject to having left over tissue destroyed. Tissue may be disposed of at the discretion of *BioCytics* and/or if requested by a trial participant at the end of the study. In circumstances where leftover tissue is used, the tissue will not be individually identifiable.

9.6. Publication of Results

Any formal presentation or publication of data collected from this trial will be considered as a joint publication by the Investigator(s) and the appropriate personnel of *BioCytics*. Authorship will be determined by mutual agreement. *BioCytics* must receive copies of any intended communication in advance of publication (at least 15 working days for an abstract or oral presentation and 45 working days for a journal submission). *BioCytics* will review the communications for accuracy verify that confidential information is not being inadvertently divulged and to provide any relevant supplementary information.

9.7. Disclosure and Confidentiality

By signing the protocol, the Principal Investigator agrees to keep all information provided in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC. Study documents provided by *BioCytics* (protocols, Investigators' brochures, CRFs and other material) will be stored appropriately to ensure their confidentiality. The information provided by *BioCytics* to the Principal Investigator may not be disclosed to others without direct written authorization from *BioCytics* except to the extent necessary to obtain informed consent/HIPAA authorization from patients who wish to participate in the trial.

9.8. Discontinuation of Study

BioCytics reserves the right to discontinue any study for administrative reasons at any time.

9.9. Data Management

9.9.1. Data Collection

Investigator(s) and/or study staff such as a clinical research coordinator must enter the information required by the protocol into the *BioCytics* CRFs. *BioCytics* monitors or designee(s) will review the CRFs for completeness and accuracy and instruct site personnel to make corrections or additions. The CRFs will be made available to *BioCytics* via EMR and EDC system(s), and ensuring all paper and electronic data be retained at the investigational site per GCP guidelines.[57] Concomitant medications and prior antineoplastic therapy (chemotherapy, radiotherapy and other) will be captured electronically in Physician Progress notes; this information can be made available upon request.

9.9.2. Data Management and Quality Control

Data items from paper and electronic CRFs will be filed in designated binders, electronic lab folders, and/or patients' electronic medical records. Carolina BioOncology Institute's EMR hosts all the clinical data for trial subjects and is developing an interface to an Electronic Data Capture (EDC) system to enable real-time collection of clinical trial data at the point of care. Subsequently this information will be systematically checked by staff during self-audits and monitoring visits. Obvious errors will be corrected by *BioCytics* or its designee personnel. Other errors, omissions, or requests for clarification will be queried; queries will be returned to the investigational site for

resolution. A copy of the signed Data Clarification Form and appropriate Protocol Deviation forms, if indicated, will be kept on file.

	Screening Visit	**Study Visits**
Time Point (Day)	**No window**	**Day 1 and Subsequent Visits[1]**
Informed Consent	x	
Enrollment and Assignment to Subject Number	x	
Centralized Reference Labs HIV, Hep. B, Hep. C, CBC, CMP, urine pregnancy test (if WOCBP)	x[5]	x[5]
ECOG Performance Status	x	x
Prior/Concomitant Anti-Cancer Treatment Documentation	x	x[2]
Pathology Report	x	
Tumor Status (NED, PD, SD, PR, CR, adjuvant remission)[3]	x	X[4]
Tumor Markers (CEA, CA19-9, PSA, CA27-29, CA15-3, CA125)	x[5]	X[5]
Blood Draw for CTC and Immune Cells	X[6]	X
Leukapheresis for CTC and Immune Cells		X[7]

10. APPENDICES

10.1. Appendix 1: Time and Events Schedule

Footnotes:

[1]visits can occur at any time from previous visit, excluding leukapheresis visits
[2]concomitant anti-cancer treatment documentation only
[3]tumor status: NED = no evidence of disease, PD = progressive disease, SD = stable disease, PR = partial response, CR = complete response
[4] Tumor Status only when indicated for standard of care or treatment purposes

[5]If clinically indicated by PI or study doctor
[6]If collecting blood for cell culture purposes, product may be quarantined if serology labs are pending
[7]Leukapheresis collection visits must occur at least 4 weeks from previous leukapheresis collection visit

10.2. Appendix 2: ECOG Performance Status[1]

ECOG Performance Status	
Grade	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Footnote:
[1] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, *et al*. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am. J. Clin. Oncol.
1982; 5: 649-655.

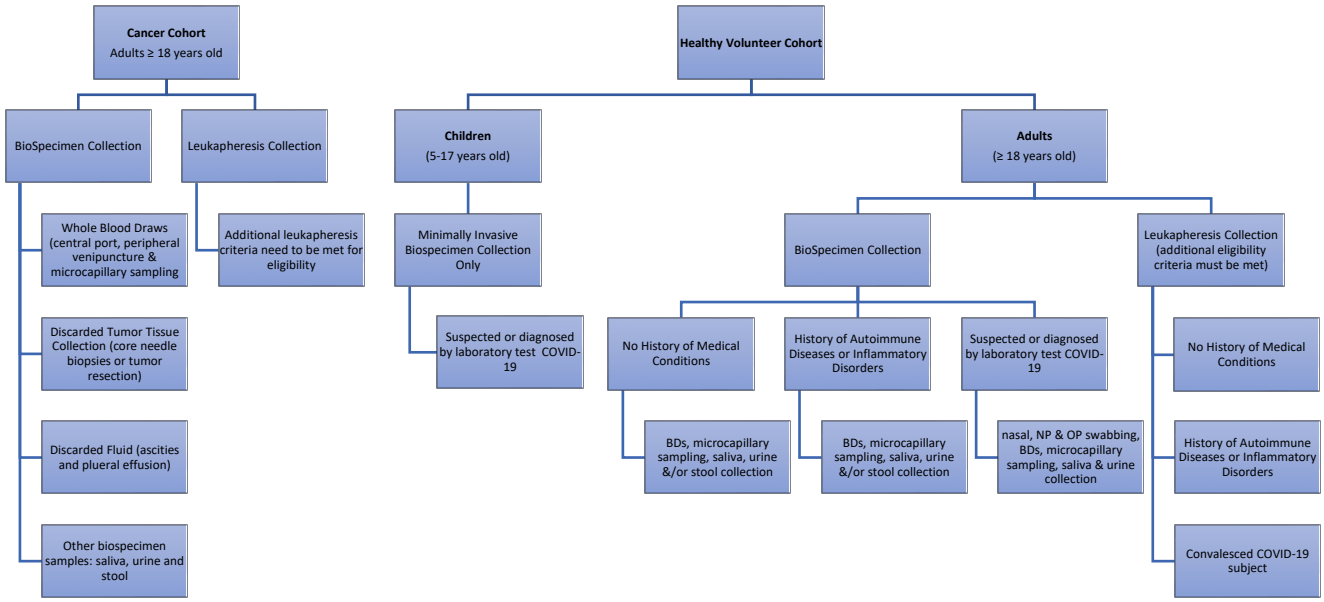
10.3. Appendix 3: First-Generation Guidelines for NCI-Supported Biorepositories
https://biospecimens.cancer.gov/bestpractices/overview.asp

10.4. Appendix 4: Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-informed-consent-vitro-diagnostic-device-studies-using-leftover-human-specimens-are-not

10.5. Appendix 5: FDA Approval of *In Vitro* Diagnostic Circulating Tumor Cell Enumeration via Immunicon Cell Tracks Analyzer II
https://www.accessdata.fda.gov/cdrh_docs/reviews/K060110.pdf

10.6 Appendix 6: FDA Guidance on Implementation on Acceptable Full-length and Abbreviated Donor History Questionnaires and Accompanying Materials for Use in Screening Donors of Blood and Blood Components (May 20, 2020)
https://www.fda.gov/media/124193/download
https://www.aabb.org/docs/default-source/default-document-library/resources/dhq-v2-1/pdfs/dhq-user-brochure-v2-1.pdf?sfvrsn=5c975155_0

10.7 Appendix 7: Cancer and Healthy Volunteer Cohort Flow-gram for Collection of Biospecimen(s) versus Leukapheresis Collection



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