PROTOCOL AMENDMENT # 9

LCCC 1108: Development of a Tumor Molecular Analysis Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

X Editorial, administrative changes (IRB Approval)

X Scientific changes (IRB approval)

Therapy changes (IRB approval)

Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY

In this amendment, some objectives have been appropriately reclassified as exploratory, and the plans for analysis have been amended to reflect the developing understanding of the study.

This amendment includes the following changes:

Editorial and Administrative Changes

- The study statistician has changed from Anastasia Ivanova to Dominic T. Moore.
- Per current practice, names of co-investigators are no longer listed.

Scientific Changes

- Section 2: Secondary objectives have been re-categorized as exploratory objectives due to this set of objectives being less informative than originally anticipated. Wording has also been clarified in Section 2.2.1.
- Section 6.1 and 6.2: Edits were made to reflect the planned analyses. These changes include deleting obsolete language from the Sample Size and Accrual language in Section 6.1, and modifying the Data Analysis language in Section 6.2 to state the Kaplan-Meier method will be used.

The attached version dated July 25, 2019 incorporates the above revisions

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PROTOCOL AMENDMENT # 8

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

X Editorial, administrative changes

X Scientific changes (IRB approval)

Therapy changes (IRB approval)

X Eligibility Changes (IRB approval)

Other

AMENDMENT RATIONALE AND SUMMARY

The amendment includes the following changes:

1. This protocol now serves as a stand-alone study in all clinics even where the Health Registry is established.
   a) The protocol still includes references to the health registry but involvement in the Health Registry is no longer required as was previously noted in the eligibility criteria. The schema in Section 4 was revised to reflect this change
2. The protocol now indicates that researchers may or may not collect normal tissue samples and they may analyze only tumor samples.
3. The protocol allows for the possible conduct of systematic germline DNA studies although this type of analysis is not required.
4. The language referring to "incidental germline discoveries" is no longer present because these discoveries are no longer incidental.
5. The protocol indicates that researchers are sequencing other genetic material besides DNA due to advancements in next generation sequencing technology (ie, RNA, ChIP, methylation sequencing, etc.).
6. Dr. Jung Wu is no longer on the list of clinical committee members and Dr. David Eberhard is no longer on the list of co-investigators.
7. Minor editorial changes are included in the text and an additional paragraph added to the background section that briefly describes NextGen Sequencing Methods.
8. Revised the description of the responsibilities of the Molecular Tumor Board in section 1.4.
9. Revised the protocol to reflect that the CCRG is no longer named the Committee for the Communication of Genetic Research Results (CCGR) but rather is now named the Clinical Committee for Genomic Research (section 1.4.1)
10. Added wording to clarify the CCGR will also use commercial or open-source products designed to evaluate the literature to the protocol (p. 36)

The attached version dated May 25, 2016 incorporates the above revisions

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PROTOCOL AMENDMENT # 7

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

X Editorial, administrative changes
Scientific changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY

The protocol is amended to include the following changes:

Personnel changes: D. Neil Hayes, MD was removed as a co-investigator and added as a Co-Principal Investigator to the study. Additionally, the study statistician was changed from Anna Snavely to Anastasia Ivanova.

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The attached version dated December 5, 2014 incorporates the above revisions

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PROTOCOL AMENDMENT # 6

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

X Editorial, administrative changes
X Scientific changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY

The driver for this amendment was to incorporate a statistical analysis plan (and revise the primary objectives accordingly) to allow researchers to estimate the proportion of patients who have a reportable genetic variant identified, and to compare clinical outcome between a subgroup of patients with and without a reportable genetic variant. In those with a genetic variant, researchers will (as a secondary objective) compare clinical outcome between those treated based on the genetic variant, and those not treated based on the variant. These major changes are reflected in section 2.0 (Study Objectives) and section 6.0 (Statistical Considerations), as well as throughout the protocol in sections 1.1 (Study Synopsis), section 1.4 (Study Overview), section 4.0 (Schema), and section 4.4 (Medical Records Abstraction).

The protocol is also amended to include the following changes:
Personnel changes: Removed Keith Amos MD from study as Co-Investigator; replaced biostatistician Pei-Fen Kuan PhD with Anna Snavely PhD; updated list of CCGR and Pathology Committee members in Appendix A.

Inserted a new section (now section 1.3) describing a prior study of use of targeted therapy based on genetic variants.

Section 1.4.1 and Appendix A describe the possibility of an additional category for gene variants.

Minor editorial changes throughout (e.g., UNCseq # of genes increased from >100 to >200); rearranged flow of background to enhance clarity

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The attached version dated May 25, 2014 incorporates the above revisions

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 5

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

x Editorial, administrative changes
___Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
x Eligibility Changes (IRB approval)
x Other

AMENDMENT RATIONALE AND SUMMARY

Protocol Cover page is updated to include Stergios Moschos, MD and Young Whang, MD, PhD as co-investigators on the study.

Section 1.2.5: Clarified that study is open to patients <18 years of age provided tissue is already stored or can be collected as part of clinically required procedure.

Section 3.1: Inclusion criteria 3.1.5 added to indicate parental consent required for any participant <18 years old, and separate consents required for minor children and adolescents.

Section 9.1: UNC Committee for the Communication of Genetic Research Results (CCGR): Updated to include Ian Davis, MD, PhD as member on CCGR for pediatric oncology.

The attached version dated March 27, 2013 incorporates the above revisions

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CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 4

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

X Editorial, administrative changes
_ Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
X_ Eligibility Changes (IRB approval)
X Other

AMENDMENT RATIONALE AND SUMMARY

The following is the summary of changes in protocol amendment 4:

i. **Throughout protocol,** Minor typos corrected
ii. **Throughout protocol**, clarified that sequencing may occur in UNC's High Throughput Sequencing Facility or other UNC research facility
iii. **Section 1.2.5 Study Overview:** Clarified that when results between UNCseq and analytically validated confirmatory testing in CLIA lab are discordant (whether the CLIA results confirm or refute UNCseq) - results from the analytically valid test only will be released to the treating physician. We also revised this section to reflect that results from confirmatory testing will be released to the treating physician regardless of the current clinical situation, provided the results can potentially inform the care of the patient.
iv. **Section 1.2.5 Study Overview and section 4.1.2 Patient Communication:** The LCCC1108 research team has implemented one additional level of oversight before results may be released to the treating physician, the Molecular Tumor Board. This is described in sections 1.2.5 and 4.1.2. We also clarified in section 4.1.2 that negative reports (that is no alteration found) will also be reported.
v. **Section 3.2.4: Exclusion criteria:** LCC1108 patients may not undergo a biopsy for research purposes only if this biopsy requires general anesthesia. This has always been the case, but is now explicitly stated in section 3.2.4., and again in section 4.3 (Expected Risks).
vi. **Section 4.1.1 Requested Studies**: Some molecular variants may be better detected via similar technologies to NextGen sequencing, including RNA sequencing. Therefore, the following statement was added to section 4.1.1: "Based on these results, additional molecular studies may be performed, if appropriate"
vii. **Sections 4.1.3.2 and 4.1.3.3** were revised to indicate that normal specimens from patients with hematologic malignancies may be obtained via skin punch biopsies under LCCC0824 as buccal swabs may be contaminated with malignant blood cells or represent an insufficient sample of DNA.
viii. **Sections 4.1.3.3 and 4.1.4:** Snap-freezing of tissue is performed by research personnel both from TPF, as well as from other clinics, so the limitation to TPF was removed.
ix. **Section 4.2:** Clarified that biopsy risks are described in the LCCC1108 informed consent
x. **Section 9.1: Appendix A: Charter:** Revised to match the corresponding changes described above for the protocol. Appendix B was removed as this is now outdated (e.g., see changed described in section 12.5 (iii) above)

The attached version dated November 28 2012 incorporates the above revisions

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 3

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

x Editorial, administrative changes
___ Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
x Eligibility Changes (IRB approval)
___ Other

AMENDMENT RATIONALE AND SUMMARY

The following is the summary of changes in protocol amendment 3:

i). Section 1.1 Study Synopsis: Editorial and clarification changes.

ii). Section 1.2: Background and Study Overview: Different subsections have been revised for clarity and consistency.

iii). Section 3.1.2, 3.1.3 and 3.1.4: Inclusion Criteria: These sections are revised for clarity. Section 3.1.4 includes another bullet point to clarify that subjects undergoing tissue collection per standard of care are willing to have additional specimen taken for the study.

iv). Section 3.2: Exclusion Criteria: Revised for clarity. Sub-Section 3.2.1 includes a bullet point to clarify that any condition that would make the participation in the protocol unreasonably hazardous for the subject in the opinion of the treating physician.

iv). Section 4.1: Study Methods and Procedures: Sub-Sections 4.1., 4.1.2, 4.1.3, 4.1.3.1-3 and 4.1.4 are revised for clarity and consistency.

v). Section 4.2: Post-collection/Follow-up Assessments: This section is revised for clarity and consistency.

vi). Sections 4.3 and 4.4: These sections are revised for clarity.

vi). Section 7.0; Sub-Sections 7.1 and 7.4: Data Management and Monitoring/Auditing: These sections are revised for clarity and consistency.

vii). Section 9.1: Appendix A: Charter: Revised for the clarity and consistency.

The attached version dated May 17, 2012 incorporates the above revisions

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 2

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

x Editorial, administrative changes
___ Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)
___ Other

AMENDMENT RATIONALE AND SUMMARY

The following is the summary of changes in protocol amendment 2:

i). Cover Page: David Eberhard,, MD, PhD is added to the study as a co-investigator. Also, Study Coordinator information is updated.

ii). Section 1.2: Study Overview: This section is updated and clarified to include information about the acquisitions of tumor samples from Health Registry/Cancer Survivorship Cohort (CSC; LCCC0906/IRB#09-0605). For the tumor samples already collected under CSC, if available, the subjects will be contacted via phone to obtain their consent for LCCC 1108.
List of reportable test is clarified to include Clinical Laboratory Improvement Amendments, CLIA-certified laboratory rather than CLIA approved equivalent.

iii). Section 3.1.2 and 3.1.3: Inclusion Criteria: These two sections are revised in order to access the tissue samples from the subjects who are deemed eligible for LCCC 1108, but their samples are either available via CSC or at another institution. Also clarified that for the tumor samples already collected under CSC or another study that include tissue banking, if available, the subjects will be contacted via phone to obtain their consent for LCCC 1108.

iv). Section 4.1.1.: Added a section on Quality Control (QC) that will be assessed by David Eberhard, MD, PhD or his qualified designee.
v). Section 4.1.4: Subsection Tissue banked at UNC: This section is revised to include facilities at UNC Chapel Hill, NC.
vi). Section 7.4: data management and Monitoring/Auditing: This section is revised for clarity on the sample tracking databases.

vii). Section 9.1: Appendix A: Charter: Revised for the clarity of test lists as applicable validation status. Also, Leigh Thorne MD and William K. Funkhouser MD, PhD are added to the Pathology Committee for LCCC 1108.

The attached version dated February 6, 2012 incorporates the above revisions

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PROTOCOL AMENDMENT # 1

LCCC 1108: Development of a Tumor Molecular Analyses Program and Its Use to Support Treatment Decisions

AMENDMENT INCORPORATES:

x Editorial, administrative changes
___ Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)
___ Other

AMENDMENT RATIONALE AND SUMMARY

The protocol is amended to include the following changes:
i). Cover Page: Robert Dixon, MD is added to the study as a co-investigator.

ii). Section 1.3: This section is updated to include information about National Institutes of Health (NIH) repository for Genome-Wide Association Studies (GWAS) for the submission of genotype and phenotype data in order to provide greater public benefit if the information is shared with a large number of researchers through the NIH repository.

iii). Appendix A: Appendix A, Clinical Committee, is revised to include David Ollila, MD to the committee as surgical oncologist. Pathology Committee is revised to include Ryan Miller, MD, PhD; George Fedoriw, MD, both from the Department of Pathology and Laboratory Medicine, UNC Chapel Hill, and Nancy Thomas, MD, PhD from the Department of Dermatology, UNC Chapel Hill. David Allan Eberhard, MD, PhD is the chair of the Pathology Committee.

The attached version dated September 2, 2011 incorporates the above revisions

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1108: Development of a Tumor Molecular Analysis Program and Its Use to Support Treatment Decisions

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Sponsor: Lineberger Comprehensive Cancer Center
Funding Source: University Cancer Research Fund, NCI Funded Grants
Version Date: July 28, 2011
Amendment 1: September 2, 2011
Amendment 2: February 6, 2012
Amendment 3: May 17, 2012
Amendment 4:November 28, 2012
Amendment 5: March 27, 2013
Amendment 6: May 23, 2014
Amendment 7: December 5, 2014
Amendment 8: May 25, 2016
Amendment 9: July 25, 2019

Signature Page

LCCC 1108: Development of a Tumor Molecular Analysis Program and Its Use to Support Treatment Decisions

Principal Investigator
H. Shelton Earp, MD

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_____

PI Signature: _____

Date:_____

Amendment 9
Version Date: July 25, 2019

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

Cancer is a disease caused by alteration of a cell's DNA sequence resulting in dysregulation of gene expression and aberrant growth. Researchers are just beginning to study the extent of DNA alterations and their consequences for patient therapy and outcomes. New technology (e.g., Next Generation (NextGen) sequencing), allows study of the spectrum of tumor genome alteration with unprecedented scope and cost-effectiveness. To be fully exploited to improve cancer care, however, it is imperative to be able to link genetic analysis to patient characteristics, treatment response and outcome.

This protocol creates options to meet these objectives either in conjunction with the UNC IRB approved Health Registry/Cancer Survivorship Cohort (CSC; LCCC0906/IRB#09-0605) or as a stand-alone study in clinics where the CSC is or is not established (and/or if patient is not eligible for or refuses enrollment in the CSC). Lineberger Comprehensive Cancer Center (LCCC) established the CSC in 2009 to create a patient registry and biospecimen repository of adult patients residing in North Carolina who receive care at the North Carolina Cancer Hospital. This important endeavor includes collection and storage of biological specimens as well as medical history and questionnaire data from all subjects enrolled. These specimens and data provide a rich resource for research studies that have IRB approval to access these data. LCCC1108 was designed in part to leverage these data, and as a complementary or stand alone study to the CSC.

The primary objectives of this study are to estimate the proportion of patients enrolled in LCCC1108 who undergo successful sequencing and have a reportable genetic variant, and to compare clinical outcomes between subgroups of cancer patients with active disease based on presence or absence of reportable variants identified by Next Generation Sequencing methods (NGS)

It is important to clarify that sequencing methodology continues to develop at a very rapid pace since the inception and implementation of this protocol and that NGS uses a wide variety of methods, allowing researchers to ask virtually any question related to the genome, transcriptome, or epigenome of a patient's tumor. Sequencing methods differ primarily by how the DNA or RNA samples are obtained (e.g., tissue type, normal vs. affected, experimental conditions) and by the data analysis options used. However, after the sequencing libraries are prepared, the actual sequencing stage remains the same regardless of the method. For example, there are a number of standard library preparation kits that offer protocols for whole-genome sequencing, mRNA-Seq, targeted sequencing (i.e., exome or 16S sequencing), sequencing of custom-selected regions, protein-binding regions, etc.

This protocol allows current or prospective cancer patients to be consented for evaluation of tumor tissue and blood samples by UNC-CH's High Throughput Sequencing Facility or other UNC research facility via NextGen sequencing (UNCseq™). The source of specimens and the methods used for molecular analysis will vary depending on the clinical situation: archival tissue (either collected under the CSC, independently of the CSC, or through another protocol). The source of specimens include research samples collected from patients already scheduled to undergo biopsy for routine clinical purposes; and research samples collected from patients willing to undergo biopsy for the purpose of research only. Investigators mayalso collect (or access, if already collected under another approved tissue banking protocol) normal germline DNA, DNA modifications, and RNA in the form of blood samples, cheek swabs, or normal tissue adjacent to tumor tissue, etc. These normal specimens may be used for molecular analysis depending on the research aim; however, analysis of tumor specimens alone may be performed and if significant reported back to the physician.

Specimens will be analyzed for genetic variants in selected genes of research interest, but only those variants of potential clinical interest will be released to the treating physician to disclose to the patient. Germline changes that are discovered and deemed of clinical interest will be reported in the context of formal genetic counseling.

1.2 Tumor Genotyping

1.2.1 Clinical Application-Current Examples

Advances in molecular genetics and genomics have enabled clinicians to minimize general treatment decisions and apply patient-specific information to treatment choice in certain situations. These advances, though currently limited in number, have had a profound impact in certain clinical settings, highlighting the fact that cancers originating from the same tissue of origin likely represent different diseases on a molecular level.[1]

The human epidermal growth factor receptor 2 protein, HER2, became one of the first biomarkers with an established role in clinical oncology, and a more patient-specific approach for treatment in cancer began with trastuzumab (Herceptin®), the humanized monoclonal antibody (MAB) targeted against HER2. Approximately 30% of breast cancers exhibit amplification of the HER2 gene, and treatments with targeted drugs that block this receptor have improved outcomes in patients with HER2 overexpressing cancers.

Agents targeted against tyrosine kinases associated with the epidermal growth factor receptor (EGFR) have an established role in the treatment of non-small cell lung cancer (NSCLC), and include erlotinib (Tarceva®) and gefitinib (Iressa®). Patients with NSCLC whose tumors exhibit mutations in EGFR, the most common of which are deletions in exon 19 and a mutation in exon 21 (occurring in approximately 10% of Caucasians and up to 50% of Asians with NSCLC) [2],

experience significantly increased sensitivity to drugs like erlotinib and gefitinib as compared to patients without these mutations.

Biomarkers are also critical in the management of metastatic colorectal cancer (mCRC), particularly related to therapy with monoclonal antibodies directed against EGFR. The RAS/RAF/MAPK pathway is downstream of EGFR, and approximately 40% of CRCs are characterized by mutations in the *KRAS* gene. These mutations predict for lack of response to the anti-EGFR MABs cetuximab and panitumumab. The relationship between these mutations and response is included in the FDA-approved labels for cetuximab and panitumumab, and use of these products is not recommended (nor are they used) in patients who carry these mutations.[3] [4]

Molecular profiling of disease may facilitate more rapid drug development and time to commercialization than current models of development. A recent example of this involves the *EML4-ALK* fusion oncogene (fusion between echinoderm microtubule-associated protein-like 4 [EML4] and anaplastic lymphoma kinase [ALK]). ALK gene rearrangements in NSCLC are always non-overlapping with mutations in EGFR, and are estimated to occur in 2-7% of non-selected NSCLC patients, and ~30% of patients when selected for adenocarcinoma, never or former light smoking history, and without EGFR mutation.[5]

Two years after the initial report of this ALK translocation, based on results of a phase I dose escalation trial, investigators reported evidence of clinical activity in NSCLC patients with this mutation treated with crizotinib, an inhibitor of ALK and MET tyrosine kinases under development by Pfizer,.[6] This finding prompted investigators to screen ~1500 tumor samples to identify 82 patients eligible for enrollment into an expanded cohort of this phase I trial, based on testing positive for ALK rearrangements.[7] Investigators reported a disease control rate of ~90% (57% response rate) in this subset, and a phase 3 registrational trial is now ongoing in ALK-positive patients, just 3 years after initiation of the phase 1 trial.[7] The dramatic results reported in early phase clinical testing of targeted therapy, such as those reported with crizotinib, prompted a recent editorial in the New England Journal of Medicine calling for accelerated approval (post-phase I testing) by the FDA of such drugs in cancer.[8]

1.2.2 Recent Developments in Tumor Genotyping

The unprecedented throughput of sequencing technologies creates opportunities to study cancer genetics on a new scale.[9] Increases in sequencing capacity over the past 6 years have been exponential and will continue to gain through the next decade. Developments in DNA technology currently permit capture of specific loci of interest from genomic DNA not only for focused re-sequencing to identify point mutations, but also for the identification of other types of lesions such as translocations, insertions, and deletions without *a priori* knowledge of the

genomic lesion. These technological breakthroughs set the stage for the next generation of clinical genetic studies of somatic mutations in cancer.

Researchers at UNC believe this new sequencing technology could allow expansion of the paradigm by analyzing all mutations and alterations in a common gene in cohorts of patients with diverse diseases. These new technologies will allow for the simultaneous, low cost analysis of >200 cancer associated genes which can then be matched with clinical data to examine the effects of these alterations on disease outcome. The sequencing methods we plan to employ will also allow for the addition of new targets for sequencing as further cancer genetic studies become available, or as sequencing capacity increases.

In addition to identifying activating mutations of oncogenes (e.g., K-RAS codon 12 mutation), new sequencing techniques can identify tumor suppressor mutations or deletions, which are more difficult to identify but of equal biologic importance. Inactivation of tumor suppressor genes is not generally assessed for clinical use at present because of technical limitations in the ability to determine these inactivating events. For the first time, this technology will allow the evaluation of how tumor suppressor mutations in cancer alter a patient's clinical course and predict response to therapy.

1.2.3 Reporting Genetic Results to Support Treatment Decisions

The reporting of results from research studies of genetic tests to treating physicians (and potentially patients) in a clinically relevant time frame varies among investigators, who are under increased pressure to report findings quickly that may have clinical significance.[10] In response to this pressure, the National Heart, Lung and Blood Institute (NHLBI) convened a working group in 2004 to develop recommendations on reporting genetic information to study subjects.[10] The panel recommended that institutions establish a standardized approach for reporting genetic results which includes a regularly updated list of tests potentially reportable to treating physicians and/or patients. According to the panel, this list should be developed and reviewed by "a group with sufficient expertise to judge the evolving scientific foundation for reporting these results."[10]

<u>Tests with Accepted Clinical Utility</u>

Most genetic tests begin under research protocols and move to the clinical setting once their use is validated for diagnosis, prognosis, or treatment.[10] In general, a biomarker is considered validated, and becomes established within routine clinical practice, if "it has proven value as a clinical test in standard practice to guide patient management"[1]. The further along a genetic test is in achieving the benchmark of validation, the more likely results of such tests will be communicated from the researcher conducting the test to the treating physician and ultimately to the patient. With canonical mutations, i.e. those validated mutations considered foundational in the development of a particular cancer or cancers, the implications for treatment and/or prognosis are well established. It is

common and appropriate to share with patients the results of tests measuring these mutations, such as those described in section 1.2.1, provided the patient desires this information.

For example, clinical practice guidelines incorporate screening for *BRCA1* mutations as well as other high-penetrance germ-line mutations in individuals at risk as part of routine standard of care. [11] [12] Testing for these mutations includes patient counseling and education prior to testing and at the time results are delivered to the patient. Incorporating these tests into guidelines is justified as results inform clinical decision making, e.g., helping to determine the surgical approach in women with newly discovered *BRCA1* related breast cancer, or offering more frequent endoscopy to individuals who carry the *MSH2* mutation to address their significantly increased risk of colon cancer.[11] Although the purpose of LCCC1108 is not to provide comprehensive screening for germline mutations, it is likely that some participants will be discovered to have an inherited cancer predisposition, in which case such information would be highly significant for the participant and for their family members.

Genetic Tests of Uncertain Significance

The role of other variants, especially shortly after discovery of their association with a particular tumor, may be of uncertain significance for cancer susceptibility, prognosis, or responsiveness to certain therapies.[11]

Until recently, it has been common practice in research studies to avoid communicating genetic test results to research subjects or their treating physicians to prevent over-interpretation of research results of uncertain clinical significance.[10] This practice has been changing recently, however, the poor prognosis and limited options available to many cancer patients are recognized, including those without an effective standard of care from the time of diagnosis (e.g., adenoid cystic carcinoma) as well as those with recurrent and progressive cancers who have exhausted all effective treatment options (i.e., candidates for many phase I trials). Thus, researchers have begun exploring ways to share critical test results when a potential clinical application exists.

A recent advance in acute myeloid leukemia (AML) illustrates the discovery of a genetic test early on in its development that could be applied clinically in the near future. Scientists at the Washington University School of Medicine in St. Louis were the first to sequence the entire genome of normal cells and tumor cells from the same patient with AML. This pivotal work lead to the discovery of somatic mutations in a particular gene (*DNMT3A*), and prompted investigators to conduct targeted DNA sequencing on close to 300 additional AML patient samples to confirm the correlation of somatic variants within this gene with treatment failure after standard dose chemotherapy.[13] While recognizing their work needed further confirmation, investigators concluded that AML patients found to have somatic variants in *DNMT3A* might need more aggressive treatment. Once these data are reproduced and clinical trials are designed to evaluate frontline dose-

intensive chemotherapy in this group, the ability to provide information to clinicians about variants in such a gene in their patients becomes critical.

1.2.4 Models of Programs that Include Sharing Genetic Results with Patients

The Massachusetts General Hospital Cancer Center (MGHCC) has recently begun promoting their efforts to share genetic results with treating physicians for rapid application into the clinic. MGHCC was the group that first reported that NSCLC patients positive for ALK rearrangements responded to the ALK inhibitor crizotinib, leading the multi-institutional collaboration that tested the drug in the expanded cohort.[7] Their Translational Research Lab (TRL) currently screens patient tumors for 120-130 specific mutations in ~15 genes. Precise robotic technology allows the TRL to process up to 100 specimens in a day, allowing for rapid genetic profiling and communication of results within a month of testing.[14] Post-testing, the treating physician informs the patient about any genetic variants identified that can be treated with targeted therapy, or that may render them eligible for enrollment into a clinical trial evaluating therapy targeted against their variant. Samples are stored so they can be re-tested if new variants (and trials designed to evaluate drugs to treat them) are discovered in the future.[14] While this system has been informative, the MGH approach employs an outdated sequencing approach based on primer extension technology. The UNC effort will identify a much larger class of variants (e.g., deletions, amplifications) in a much larger number of genes (>200) using Next Generation sequencing techniques.

Another interesting model for rapid genetic profiling specifically geared towards clinical application can be found at the Clearity Foundation (www.clearityfoundation.org). The mission of this foundation is to improve treatment outcomes in recurrent and progressive ovarian cancer by providing molecular analysis of tumor specimens upon request from physicians. Through partnership with CLIA-certified labs, the foundation offers access to molecular tests provided formalin-fixed paraffin-embedded (FFPE) tumor blocks, unstained slides, or fresh frozen tissue specimens are available. The panel of tests they run includes biomarkers targeted by commercially available and investigational drugs, as well as biomarkers indicating sensitivity or resistance to certain chemotherapy. Test results are provided as soon as 7 days after receipt of the sample, with full reports provided in 4 weeks. They have been providing this service for almost three years. The Foundation also offers profiling of newly diagnosed patients not for immediate application, but in the event the patient recurs in the future.

Patients have brought results of their genetic tests from outside laboratories to UNC clinicians, including tests conducted in the Clearity Foundation partner labs, but concerns exist surrounding the application of these results to guide clinical care. While the Scientific Advisory Board of this foundation includes high-level representatives from respected institutions in the field of oncology (e.g. Memorial Sloan Kettering, Dana Farber Cancer Institute, etc.), relying on this type of testing with uncertain validation could be problematic. From a review of the tests offered

via Clearity Foundation's website, the investigators at UNC-CH would not consider many of these tests to be of accepted clinical utility.

While very little data exist regarding communication of individual genetic results to cancer patients participating in trials, results to date are encouraging. A recent pilot study evaluated the impact of sharing results of the germline *CDKN2A* mutation in a subset of melanoma survivors who had participated in a Genetics, Environment and Melanoma (GEM) study at the University of Michigan hospital. This mutation confers a risk of 30-65% through age 80 for development of melanoma, and a 5% risk for another melanoma among melanoma survivors.[15]

The majority (70%) of people enrolled in the pilot trial (n=19 of 27 contacted) opted to be told whether they carried a *CDKN2A* mutation, and investigators reported the knowledge did not cause the participants emotional distress (measured 1 week and 3 months after disclosure) or change their health behavior.[15] Of note, subjects received educational materials about risk factors for melanoma and reviewed these materials with a genetic counselor prior to disclosure of their mutational status by the genetic counselor.[7] In addition, most participants indicated an increased willingness to participate in genetic research in the future because of disclosure. Of note, LCCC1108 focuses on communication of genetic results from somatic profiling of tumor tissue, and does not focus on germline genetic profiling. Germline changes discovered and deemed clinically relevant, however, will be reported to patients in the context of formal genetic counseling.

1.3 Using Genotyping to Treat: a Prospective Pilot Study

Treating patients based on genetic variants, a so-called "genome-forward approach", has been applied across tumor types, with clinical benefit reported in a prospective pilot study. Von Hoff and colleagues published a multicenter, single arm pilot study of patients with a wide variety of refractory cancers that utilized molecular profiling to select "targeted" treatment.[16] Molecular targets were detected via oligonucleotide microarray (MA) gene expression assays on fresh (frozen) biopsy tissue, and via immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) on formalin-fixed tissue. A list of possible therapies based on targets was generated based on an extensive review of the literature and using data from a prior retrospective feasibility study. These agents included those that are traditionally considered targeted against a particular molecule (e.g., trastuzumab for HER2/Neu) as well as those not traditionally considered targeted drugs (e.g., irinotecan for carboxylesterase (CES-2)). In this pilot study of 86 patients, 66 were treated according to molecular results.[16]

In terms of efficacy, patients served as their own control; progression-free survival (PFS) post "molecularly-targeted" treatment (PFS-MT) was compared within individual patients to PFS after their most recent regimen (PFS-RT). Of the 66 patients treated according to molecular results, 27% had a PFS ratio of ≥ 1.3 (PFS-MT/PFS-RT).

Researchers also documented the choice of therapy the treating physician would have chosen without molecular results available, and none of them matched the targeted therapy.[16]

1.4 LCCC1108: Study Overview

Patients with a diagnosis or potential diagnosis of cancer will be consented for enrollment into this study. In clinics in which the IRB-approved Cancer Survivorship Cohort (CSC, LCCC0906) is operating, eligible patients for LCCC1108 may be offered enrollment into the CSC via the CSC global consent if they are eligible for LCCC0906. In clinics in which the CSC is or is not yet operational and/or the patient is not eligible (or has declined LCCC0906 participation), LCCC1108 will function as a stand-alone protocol. Patients deemed eligible for LCCC 1108 who may have been offered the opportunity to enroll into CSC prior to LCCC1108 initiation) and who have stored tumor tissue samples in the Tissue Procurement Facility (TPF) may be contacted via telephone for enrollment into LCCC1108 and to obtain consent for research access to their stored tumor tissue.

Specimens collected under LCCC1108 consent will be stored in the TPF along with any specimens previously collected as part of routine clinical care and stored under the general TPF banking protocol (LCCC9001), under the CSC global consent, under this protocol, or under another approved tissue banking protocol for consented participants. Genomic DNA or RNA will be obtained either as part of the CSC (and handled and stored by the Biospecimens Core), or under LCCC1108. Appropriate tissue and possibly germline DNA and RNA will be released for molecular analysis to UNC's High Throughput Sequencing Facility or other UNC research facility. Medical records will be abstracted to facilitate correlation of clinical data with genetic testing in studies using the mechanisms approved for the CSC.

It is expected that most LCCC1108 participants will provide samples through procedures that are part of their standard clinical care, and thus, the additional risk of providing tissue to be used in this research will be minimal. Many participants will already have tissue and/or blood specimens banked in TPF under the general TPF protocol (LCCC9001) or under another approved tissue banking protocol. Others, who are consented to LCCC1108 pre-operatively, will allow any surplus tissue and/or blood (after the standard diagnostic procedures have been completed) to be accessed, stored, and processed for the research described herein. Parental consent is required for all patients <18 years old. Separate consent documents are also required for minor children (7-14 years old) and adolescents (15-17 years old). Any participant <18 years of age must have tissue already banked, or be undergoing a procedure as part of their standard clinical care.

A subset of patients ≥18 years of age will be asked to submit tissue and blood specimens obtained from procedures without a specific identified clinical or diagnostic need, based on current clinical standards of care. Biopsies of vital organs (e.g., lung, liver etc.) will be restricted to those participants ≥18 years of age whose treatment offers no expectation of cure, such as patients with refractory solid tumors who relapse with metastatic disease, e.g. Other biopsies performed outside of clinical standard of care will be limited to those tumors that can be accessed without subjecting the participant to unreasonable risk, as determined by the physician performing the procedure.

Specimens will be analyzed for alterations in a panel of specific genes of cancer relevance, but only genetic variants of potential clinical interest will be released to the treating physician to disclose to the patient. Germline changes that are discovered by systematic analysis and deemed clinically relevant will be reported in the context of formal genetic counseling. Results of genetic tests from the "gene list" will be analytically validated in a CLIA-certified laboratory prior to distribution to the treating physician (see possible exception to this requirement noted below). Note: when results between NextGen sequencing and CLIA are discordant, results from the CLIA-certified laboratory only will be distributed to the treating physician.

LCCC1108 Molecular Tumor Board (MTB) reviews the sequencing information and identifies the genes of relevance based on CCGR recommendations to move forward for clinical confirmation. This tumor board provides additional oversight, ensuring compliance with the protocol, technical quality assurance, and appropriate application of results to individual patients. This board is comprised of representatives of the CCGR, the Pathology committee, key laboratory scientists involved in technical and bioinformatic aspects of UNCseq™, and clinical/molecular pathologists.

The sequencing data and outcomes from the CLIA confirmation assays are reviewed in aggregate to ensure quality assurance measures are being met. Our confirmation rate historically is greater than 95%.

1.4.1 UNC Clinical Committee for Genomic Research (CCRG)

The UNC CCGR has been established per the NHLBI recommendations cited earlier.[10] This group will meet quarterly to review and update the list of reportable tests, and in what setting each test would be clinically relevant based on its potential for clinical application. See Appendix A for the CCGR Charter. In brief, the Committee will categorize reportable tests into 3 lists:

- **List #1**: Genetic alterations of accepted clinical utility (and validated (whether confirmed or refuted) in a CLIA certified laboratory) used in standard of care (SOC) management of patients (e.g., KRAS in mCRC)

- **List #2**: Genetic alterations of potential clinical significance (and validated (whether confirmed or refuted) in a CLIA certified laboratory for use in non-SOC settings (e.g., EGFR in breast cancer)
- **List #3:** Genetic alterations of potential clinical significance (without the possibility of validation in a CLIA certified laboratory for use in non-SOC settings (e.g., LKB1)

As of the writing of Amendment #6, a List #4 is under consideration by the CCGR. This list would include genetic variants that predict sensitivity to non-targeted treatment (e.g. radiation, cytotoxics).

In developing the lists, the Committee will receive input from the LCCC Oncology Disease Groups (e.g., Breast Cancer, Lung Cancer) and the LCCC1108 Pathology Committee (see Appendix A). For List #3, when validation in a CLIA certified laboratory is not available or possible, results of NextGen sequencing will be provided to the patient through the treating physician on a "compassionate use" basis, but only if the following criteria are met (which we anticipate will be rare):

- The UNC Committee deems the information would be of clinical interest
- The patient has advanced cancer for which there is no standard of care
- Results are reproduced in the research laboratory using NextGen sequencing
- The results include a statement that "This is a research result from a non-validated test environment"
- A waiver for the CLIA-certification requirement is obtained from the UNC IRB on a case-by-case basis.

Similar to the program established at MGHCC, we will not share tests performed at UNC with treating physicians unless they can potentially inform the care of a particular patient disease population.

1.4.2 Treatment Decisions

Ideally, reportable findings in patients with refractory disease (after confirmation by validation in a CLIA certified laboratory) will be used to influence the choice of a clinical trial when standard of care options are exhausted (e.g., identification of a PI3K mutation may lead the treating physician to choose a trial that includes a PI3K inhibitor if the patient meets eligibility criteria). Clinical trials may not be available in all such patients, however. Therefore, in patients for whom there is no available trial, the finding of an unexpected, but targetable variant could lead to the off-label use of an approved therapy. For example, a small percentage of patients with ovarian cancer will be found to harbor activating KIT mutations, and such patients could be treated with anti-KIT agents (e.g. dasatinib) based on results of NextGen analysis (with re-testing in a CLIA certified laboratory) even though dasatinib is not presently approved for use in ovarian cancer.

Use of results of the UNCseq™ intervention from the "Gene List" as the basis for treatment or to refer a patient to a clinical trial, will be up to the discretion of the treating physician,. Treatment decisions will be documented via use of the UNCseq™ Physician survey (an IRB-approved survey provided separate from this protocol) and review of medical records. If treatment is based on molecular testing, the specific treatment (including referral to a clinical trial, or choice of commercial agent(s), dose and duration) will be documented when this information is available.

It is expected that any therapy chosen based on this list by the treating physician will be carried out using the most current prescribing information for that therapy available from the FDA, and/or based on a review of the scientific literature for that particular tumor type. Outside of a clinical trial, we anticipate that most physicians will evaluate tumor response every 6-8 weeks (or other as per standard of care for the particular tumor type) as they would when starting any patient on a new anti-cancer therapy. These data will be recorded, as will the date of progression (if available) and patient survival.

1.4.3 Characterization of Population and Treatment Outcomes

While several attempts have been made to document the proportion of specific genetic alterations in selected Phase I populations, the intervention in LCCC1108 will provide the opportunity to characterize the proportion of most of the known clinically relevant genetic alterations across a wide variety of tumors. [17-19] Specifically, as a primary objective, we will estimate the proportion of enrolled patients who have undergone successful sequencing and who have a reportable genetic variant identified via UNCseq™. The study intervention, includes the use of highly and ever evolving sequencing methods and genomic technologies that may be applied to both somatic and germline tissues, and correlated with extensive clinical annotation and outcome data. These technologies may associate known molecular alterations in a variety of tumors with prognosis and/or clinical outcomes. A primary objective of this study is to compare PFS ratios between cancer patients with active disease with a reportable genetic variant and those without a reportable genetic variant. We chose the PFS ratio as it is the approach already used and published by Von Hoff, and it allows the patient to serve as his or her own control. Whether disease is defined as active or not takes place at the time of the MTB meeting. In this context, a patient is considered to have active disease provided they have not been declared to have NED (no evidence of disease) and they are not in hospice. The PFS ratio is defined as the PFS after the first treatment post UNCseq™ results divided by the PFS post the most recent therapy prior to UNCseq™on which the patient has just experienced progression. See section 4.4 for additional details on this endpoint.

Using clinical data from patients enrolled in this protocol, either through LCCC0906 (the CSC) or LCCC1108, we plan to facilitate explorations of any correlation between these genetic alterations and response to molecularly based treatment under this and future IRB-approved protocols. As a secondary

objective, we will compare PFS ratios between patients with active disease and a reportable genetic variant who were treated based on this variant versus those not treated based on this variant (see section 4.4).

1.5 Rationale

By sequencing targeted regions of a subject's tumor genome, and returning relevant and individual results to the treating physician, we hope to provide a mechanism for rapid, patient-specific treatment decisions. These data will become part of the patient's information in the CSC database if they have consented to be part of that study.

The number and type of situations appropriate for patient-specific approaches to cancer treatment are expected to grow as scientists, including those at UNC, discover new molecular alterations across a broader range of tumor types. These discoveries are essential, as traditional approaches to treatment have made limited progress over the past several decades, and virtually none against advanced solid tumors.[20]

Patient samples and/or data from this repository may also be submitted to the National Institutes of Health (NIH), National Cancer Institute (NCI) repository for the purposes of Genome-Wide Association Studies (GWAS).

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To estimate the proportion of patients enrolled in LCCC1108 who have undergone successful sequencing (see section 6.0) and have a reportable genetic variant identified via UNCseq™

2.1.2 To estimate 2 year PFS in cancer patients with active disease (defined in section 1.4.3) with a reportable genetic variant and those without a reportable genetic variant

2.2 Exploratory Objectives

2.2.1 To estimate differences in PFS between cancer patients with active disease with a reportable genetic variant who were treated based on variant, versus those who were not treated based on a variant.

2.2.2 To collect and describe clinical data (e.g., medical history, demographics) including treatment outcomes after availability of UNCseq™ results in patients

2.2.3 To link tumor samples and other biospecimens for future IRB approved clinical trials that have a tissue specimen component

2.3 Primary Endpoints

2.3.1 The proportion of those patients with successful sequencing (see section 6.2) who have any non-standard of care variant discovered via UNCseq™ and confirmed after UNCseq™ in a CLIA certified laboratory (or provided by a waiver by the IRB).

2.3.2 PFS is defined as the time from day 1 of treatment until disease progression or death as a result of any cause.

2.4 Exploratory Endpoints

Other efficacy outcomes that may be evaluated include objective response (OR), stable disease (SD) and overall survival (OS), and will be defined as per RECIST1.1 or per the appropriate hematologic disease guidelines [21-26]. Treatment decisions using UNCseq™ are based on the treating physician's responses to the UNCseq™ Physician survey (an IRB-approved survey provided separate from this protocol).

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

Patients must fulfill all criteria to be eligible for this study:

3.1.1 Current or prospective cancer patients; current cancer patients must have histologically or cytologically confirmed diagnosis of cancer

3.1.2 Patients who are considering enrollment in LCCC1108, who are eligible for the CSC, and who are seen in a clinic at UNC in which the UNC Health Registry/CSC is operational may be offered enrollment into the CSC via the CSC global consent; however participation in the CSC is optional and patients can enroll in LCCC1108 alone. Patients deemed eligible for LCCC 1108 who have enrolled into CSC or another study that included banking of tumor tissue prior to LCCC1108 initiation (and who have tumor tissue already stored) may be contacted via telephone for enrollment into LCCC1108 for access to their stored tumor tissue. Telephone consenting will be documented properly per IRB guidelines.

3.1.3 Participant must have tumor tissue available and suitable for molecular analysis from at least one of the following sources:

- Tissue previously stored at UNC, Chapel Hill, NC (e.g., tissue collected under the CSC (IRB # 09-0605), IRB # 01-1283, IRB #90-0573, IRB#09-0768, or IRB #05-2015)

- Tissue previously stored at an outside institution (other than UNC-CH), provided investigators can determine that the tumors were sampled and stored under appropriate conditions for inclusion in LCCC 1108 study
- Patient is undergoing tissue collection as per clinical standard of care and is willing to allow specimens from surplus tissue to be diverted for research purposes
- Patient is undergoing tissue collection as per clinical standard of care and is willing to have additional specimens taken for research purposes
- Patient is willing to undergo biopsy to procure tissue samples for research purposes only without a previously identified clinical or diagnostic need

3.1.4 For those ≥ 18years old capable of providing informed consent and willing to sign the IRB-approved written informed consent agreements for the present protocol

3.1.5 For minor children 7-14 years of age, and adolescents 15 to 17 years of age, capable and willing to provide informed consent; parental consent also required.

The following additional inclusion criteria apply only to patients undergoing biopsy for research purposes only under this protocol (i.e., without a previously identified clinical or diagnostic need):

3.1.6 ≥18 years of age

3.1.7 Treatment options offer no expectation of cure (e.g., advanced solid tumor patients with metastatic disease) **NOTE:** This restriction applies to biopsy of vital organs only (e.g., lung, liver)

3.1.8 Appropriate candidate for research biopsy based on institutional standards for target biopsy site

3.2 Exclusion Criteria

Patients who fulfill any of the following criteria will be excluded:

3.2.1 Any condition that would make participation in the protocol unreasonably hazardous for the patient in the opinion of the treating physician

3.2.2 Affected by dementia, altered mental status, or any psychiatric or co-morbid condition that would prohibit the understanding or rendering of informed consent

The following additional exclusion criteria apply only to enrolled patients undergoing biopsy for research purposes only:

3.2.3 History of serious or life-threatening allergic reaction to local anesthetics (i.e. lidocaine, xylocaine) or any medications used for conscious sedation (if applicable)

3.2.4 Requires general anesthesia for collection of biopsy

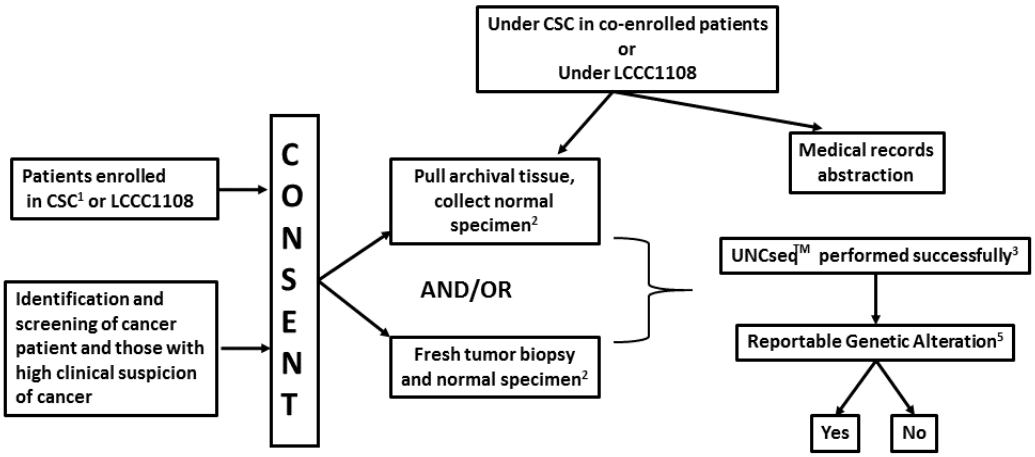
3.2.5 Currently pregnant or lactating

3.2.6 Active cardiac disease, defined as:

a. History of uncontrolled or symptomatic angina.
b. History of uncontrolled arrhythmias
c. Myocardial infarction < 3 months from study entry
d. Uncontrolled or symptomatic congestive heart failure
e. Any other cardiac condition, which in the opinion of the treating physician would make this protocol unreasonably hazardous for the patient

3.2.7 Patients receiving bevacizumab less than 6 weeks prior to enrollment into this study should not undergo research core biopsies because of the concern for potential increased bleeding risk and delayed healing. (**NOTE**: Patients receiving bevacizumab who are undergoing a research biopsy of accessible organs (e.g. breast, lymph node, skin etc.) must be two weeks from the last dose of the angiogenesis inhibitor).

4.0 **Schema**



1. Patients may be offered enrollment in UNC Health Registry/Cancer Survivorship Cohort (CSC) if eligible and CSC is operational in their clinic or offered enrollment in LCCC1108 alone.
2. Normal specimen may be collected adjacent to tumor, blood sample, buccal swab etc. but this is not required.
3. See section 6.0 for definition of successful sequencing. The PFS will be documented in cancer patients with active disease (see section 1.4)

4.1 Study Methods and Procedures: Screening and Informed Consent

During the informed consent process, patients will be informed that results of some (but not all) of the genetic testing on their tumor specimens may be communicated directly to them through their treating physician. Along with the results that are communicated, the participant's treating physician will explain the implications of their testing results, including whether their genetic profile renders them eligible for a specific therapy or clinical trial, and/or if their genetic profile carries any prognostic significance for their disease. Participants will be informed that any specimens remaining after genetic profiling of their tumor to support their clinical care is complete, will be stored indefinitely and may be used for future unspecified research related to cancer. This may include development of cell lines or commercial products from their specimens.

We will inform patients that researchers may contact them in the future to request their participation in new research or in the event new discoveries or therapies become available that may be applicable to their specific genetic profile. We recognize that some patients may transfer their care to an outside institution. In the event a participant moves or transfers his or her care to an outside institution, he or she will be asked to contact the Study Coordinator to provide information about his or her disease status.

As is standard with all trials that may incorporate germ-line studies, we will inform patients of the potential implications for family members and the risks associated with such studies, including those related to insurability and employment and the limitations of laws designed to protect against such risks. However, it will be made clear to patients that although a blood sample is being obtained that may be used for comparison to their germline genome sequence, this study is not aimed at discovering germline mutations. We will emphasize that our analyses should not be construed as genetic testing for genetic mutations associated with hereditary cancer susceptibility. Nevertheless, because of the methods used, it is possible that we will discover a germline mutation that predisposes to cancer, in which case this information will be conveyed to the participants and their families through standard genetic counseling.

4.1.1 Requested Studies

Researchers at UNC's High Throughput Sequencing Facility (or other UNC research facility) will perform molecular studies on genes of relevance to cancer. DNA and/or RNA isolation will be performed under the direction of Dr. Xiaoying Yin. Genomic data analysis will be conducted under the direction of Dr. D. Neil Hayes. Based on these results, additional molecular studies may be performed, if appropriate. Only variants of clinical interest, as determined by the UNC CCGR, will be conveyed to the treating physician (see Appendix A). If possible, results of reportable genetic tests will be validated (whether confirmed or refuted) analytically in a CLIA-certified Laboratory prior to distribution to the treating physician. NOTE: See Section 1.2.5 for possible exceptions to the CLIA requirement.

Quality Control (QC) of Tissue Samples

Prior to molecular analysis of samples accessed under this protocol, it is essential that QC be performed by qualified personnel to ensure that the case diagnosis and tissue histopathology are adequately represented to permit successful DNA and RNA sequencing. A qualified person will perform this QC prior to molecular analysis.

4.1.2 Patient Communication

Results will be placed in the patient's medical record if confirmed by a CLIA-certified laboratory and signed off by the MTB. Notification of results of reportable genetic profiling will be provided to the patient's treating physician who will be responsible for sharing results with their patient. Negative reports (i.e. no alteration found) will also be reported. If a germline mutation is discovered that has clinical implications with respect to hereditary cancer predisposition, for example, this information will be conveyed through formal genetic counseling. Along with the results, the participant's physician will explain the implications of the testing, including whether their genetic profile renders them eligible for a specific therapy or clinical trial, and/or if their genetic profile carries any prognostic significance for their disease. Participants will be informed that researchers may contact them in the future, to request their participation in new research or in the event new discoveries or therapies become available that may be applicable to their specific genetic profile.

4.1.3 Normal Specimen Samples

NOTE: If the patient is co-enrolled in LCCC0906 (the CSC), LCCC0824 (IRB#09-0768), LCCC0121 (IRB#01-1283), and/or other specimen protocols that include collection and storage of normal specimens in the TPF, these samples may be accessed if sufficient quantities are available, if samples are of the appropriate type, and if distribution is approved by the PI of the study. The following sections apply to patients who are not co-enrolled into one of these protocols.

4.1.3.1 Blood Samples

During screening, potential study subjects should be queried about any medications they are taking (see Eligibility Criteria). If applicable, a pregnancy test (blood or urine) should be done. For patients undergoing biopsy, the physician performing the procedure will ensure the patient meets all institutional standards for the procedure, and may collect ~8mLs in a single yellow top ACD tube (i.e., collection of normal tissue is not required). Alternatively, the participant can have a blood sample obtained during pre-operative care or in the outpatient phlebotomy clinic as appropriate. Inpatient participants may have blood samples taken as part of their regular phlebotomy draw.

For eligible patients who are not undergoing biopsy, a single yellow top ACD tube may be collected (~8 mLs of blood) for storage within 28 days of consent (if

possible), unless the investigator deems another source of normal DNA (or RNA) is more appropriate (see below).

For storage, serum will be processed by and stored in TPF, with each aliquot assigned a unique identification code, as per standard TPF procedures. Genomic DNA (or RNA) may be obtained and appropriate germline DNA (or RNA) may be released to the High Throughput Sequencing Facility or other UNC research facility for molecular analysis.

4.1.3.2 Buccal (Cheek) Swab Collection

For patients in which blood sample collection is not obtained, we may obtain two buccal swabs from the inner cheek to obtain germline DNA. DNA will be isolated from the swabs, frozen, stored in the TPF, and given a unique identification code, as per standard TPF procedures. Genomic DNA may be obtained and germline DNA may be released to the High Throughput Sequencing Facility or other UNC research facility for molecular analysis.

4.1.3.3 Other

The collection of normal tissue is not required. However, normal specimens may also be obtained from other sources, such as normal tissue adjacent to tumor during the biopsy procedure, or via a skin punch biopsy in patients with hematological malignancies (note: skin punch biopsies for hematological patients will be collected under LCCC0824). Normal specimens adjacent to tumor tissue collected during a biopsy procedure and skin punch biopsies will be immediately snap-frozen by research personnel using standard procedures, stored in the TPF facility in a -70°C freezer, and given a unique identification code. Genomic DNA (or RNA) from the tumor and the germline sample maybe released to the High Throughput Sequencing Facility or other UNC research facility for molecular analysis.

4.1.4 Tumor Biopsy Collection

<u>Research Biopsies</u>

In order to minimize the risk of a tissue procurement procedure (biopsy, thoracentesis, or paracentesis, e.g.), only qualified personnel will perform these procedures. Prior to the procedure, the physician performing the procedure will ensure the patient meets institutional standards for the procedure, discuss the risks with each study participant, answer any questions, and obtain separate procedure consent.

For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. Under sedation, biopsies will be made with an 11g -18g core needle depending on site and judgment of the physician performing the biopsy, with a maximum of 4-6 passes through tumor made if this is felt to be safe by the physician performing the biopsy procedure. Alternatively, surgical excision of superficial sites of disease is also appropriate if

preferable to the patient over needle biopsy. After lymph node biopsies, patients will be observed for approximately 2 hours (range 2-4 hours) after the procedure, or per institutional standard guidelines. After liver biopsies, patients will be observed for approximately 4 hours (range 4-6 hours) after the procedure, or per institutional standard guidelines.

Biopsies will be immediately snap-frozen by research personnel using standard procedures, stored in the TPF facility in a -70°C freezer, and given a unique identification code. Genomic DNA or RNA will be obtained and appropriate tissue DNA or RNA released to the High Throughput Sequencing Facility or other UNC research facility for molecular analysis.

Biopsy/Surgery as Part of Routine Clinical Care

Tumor biopsy samples and surgical specimens will also be eligible for genotyping in patients scheduled to undergo biopsy and/or surgery as part of routine clinical care. In this situation, patients will be consented to have additional specimens taken for research purposes only, and/or to have any excess tissue remaining after clinical purposes are satisfied analyzed for molecular alterations (via LCCC0906, if possible). Researchers will ensure that the tumors were appropriately sampled and stored for inclusion in this study. If a blood, buccal sample, or other normal specimen is not collected during the procedure, patients may be asked to provide one as soon as practicable (see 4.1.3).

Tissue Banked at UNC

If a patient consenting for enrollment into this protocol has had tissue and blood stored in the TPF or other UNC-CH facility from a prior procedure, we will request their consent for access to their stored specimen(s). If a blood or buccal sample or other normal specimen was not collected, patients may be asked to provide one as soon as practicable (see 4.1.3).

4.2 Post-collection/Follow-up Assessments

Post-procedure observation will be per the standard of care for the particular anatomic site to be biopsied. For example, patients undergoing liver biopsy will either be observed for approximately 4-6 hours in the interventional radiology suite (PRU-Procedural Recovery Unit, 2nd floor Main Hospital) or held for overnight observation at the discretion of the physician performing the biopsy, or per institutional standard guidelines (also in the PRU).

For biopsies performed for research purposes only, patients will be seen by one of the study investigators one to two weeks after the biopsy to rule out any late complication thereof. Complications will be recorded as adverse events using NCI CTCAE version 4.0 and Serious Adverse Events will be reported as described below. For clinically indicated biopsies, follow-up and recording of adverse events will be completed as per standard of care by the treating physician for the particular anatomic site to be biopsied.

4.3 Expected Risks

Pain or discomfort related to the procedure is minimized with the use of sedation. Patients who require general anesthesia to undergo a separate biopsy procedure for research purposes only are not eligible for LCCC1108.

Biopsy Risks

Potential adverse effects of biopsy include bleeding (which can necessitate a blood transfusion and/or hospitalization, and can be life-threatening) and infection, both with very low frequencies. Bleeding at the time of biopsy will be controlled by standard clinical procedures. Damage to local structures will be minimized by knowledge of local anatomy and standard pre-biopsy imaging studies. Other risks related to biopsies are dependent on the site of the biopsy. These are described in the LCCC1108 informed consent.

Local Anesthesia

With regard to anesthesia, all biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation

Certain biopsy procedures, such as lymph node or liver biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain continuously a patent airway independently and respond appropriately to physical stimulation and verbal commands.

The medications used to induce conscious sedation include the benzodiazepine midazolam and the opioid agonist fentanyl, or as per standard of care. IVCS is performed once over a 30-60 minute period, and may require administration of multiple doses of each agent over this time frame. Rarely, IVCS will last longer than 60 minutes.

Midazolam:

See http://www.drugs.com/pro/midazolam-injection.html for complete prescribing information on midazolam, including complete information on risks associated with its use.

The risks of midazolam include respiratory depression and respiratory arrest, especially when used for sedation in non-critical settings. Respiratory arrest could require intubation. In some cases, when not recognized promptly and treated effectively, death or hypoxic encephalopathy has resulted. Other serious cardiorespiratory adverse events have occurred after administration of midazolam, including airway obstruction, oxygen desaturation, apnea, and cardiac arrest, sometimes resulting in death or permanent neurologic injury. There have also been rare reports of hypotensive episodes requiring treatment, particularly in

patients with hemodynamic instability. Agitation, involuntary movements, hyperactivity and combativeness have been reported in adult patients treated with midazolam.

Concomitant use of midazolam with other respiratory depressants like fentanyl may increase the risk of hypoventilation, airway obstruction, desaturation, or apnea, and may contribute to profound and/or prolonged drug effect. Prolonged sedation may also be seen when midazolam is administered concomitantly with drugs known to inhibit the P450 3A4 enzyme system such as cimetidine, erythromycin, diltiazem, verapamil, ketoconazole, and itraconazole.

Adverse effects reported after intravenous administration of a single dose when used as a sedative include the following (Note: percentages given represent the fraction of all reported adverse events in adults): hiccoughs (3.9%), nausea (2.8%), vomiting (2.6%), coughing (1.3%), "oversedation" (1.6%), headache (1.5%), and drowsiness (1.2%). In addition, the following local effects at the site of the injection have been reported: tenderness (5.6%), pain during injection (5.0%), redness (2.6%), induration (1.7%), and phlebitis (0.4%). Additional rare (<1.0%) adverse events occurring when midazolam is used as a sedative include:

Respiratory **(**laryngospasm, bronchospasm, dyspnea, hyperventilation, wheezing, shallow respirations, airway obstruction, tachypnea);

Cardiovascular (bigeminy, premature ventricular contractions, vasovagal episode, bradycardia, tachycardia, nodal rhythm);

Gastrointestinal (acid taste, excessive salivation, retching);

CNS/Neuromuscular (retrograde amnesia, euphoria, hallucination, confusion, argumentativeness, nervousness, anxiety, grogginess, restlessness, emergence delirium or agitation, prolonged emergence from anesthesia, dreaming during emergence, sleep disturbance, insomnia, nightmares, athetoid movements, seizure-like activity, ataxia, dizziness, dysphoria, slurred speech, dysphonia, paresthesia);

Special Senses (blurred vision, diplopia, nystagmus, pinpoint pupils, cyclic movements of eyelids, visual disturbance, difficulty focusing eyes, ears blocked, loss of balance, light-headedness);

Integumentary (hive-like elevation at injection site, swelling or feeling of burning, warmth or coldness at injection site);

Hypersensitivity (allergic reactions including anaphylactoid reactions, hives, rash, pruritus);

Miscellaneous (yawning, lethargy, chills, weakness, toothache, faint feeling, hematoma).

Fentanyl

See http://www.drugs.com/pro/fentanyl-injection.html for complete prescribing information on fentanyl (Duragesic®), including complete information on risks associated with its use.

Fentanyl may cause muscle rigidity, particularly involving the muscles of respiration. Skeletal muscle movements in the extremities, neck and external eye have also been reported with fentanyl; rarely, these have been strong enough to pose patient management problems. Fentanyl may also produce euphoria, miosis, bradycardia and bronchoconstriction, as seen with other narcotic analgesics. The most common serious adverse reactions reported with fentanyl include respiratory depression, apnea, rigidity, and bradycardia. If these remain untreated, respiratory arrest, circulatory depression, or cardiac arrest could occur. Other adverse reactions that have been reported are hypertension, hypotension, dizziness, blurred vision, nausea, emesis, laryngospasm, and diaphoresis.

The risks of IVCS also include allergic reactions to the sedative or analgesic medications and inhibition of the gag reflex and concomitant risk of aspiration. The chance of serious risks from IVCS are small but real; for example, a recent retrospective evaluation of 324,737 patients sedated using opioids and benzodiazepines for endoscopic procedures reported in the Clinical Outcomes Research Initiative of the American Society for Gastrointestinal Endoscopy reported 39 deaths (11 per 100,000), including 28 cardiopulmonary deaths (8 per 100,000) (Sharma et al, Gastrointest Endosc 2007;66:27-34).

In order to minimize the risk of IVCS, only qualified, licensed personnel (M.D. and R.N.) will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any necessary supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place (in the interventional radiology suite, e.g.) will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway, should this be necessary. An emergency cart will also be immediately accessible, and emergency support services will be available. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. Following the procedure, patients will be observed closely in the recovery room according to standard institutional guidelines.

Risks of Blood Draws and I.V. Insertion

Blood draws may be associated with slight discomfort from the needle-stick, localized erythema, bleeding/bruising, or soreness around the area where the needle is inserted. Insertion of an intravenous catheter (I.V.) has similar risks, in addition to a small risk of infection at the site where the needle is inserted.

In order to minimize the risk of blood draws and I.V. insertion, only trained personnel will perform these procedures, according to standard institutional guidelines.

Risks of Imaging Studies

Some biopsy procedures require imaging studies, either to plan or guide the procedure. Imaging studies that may be used in obtaining tissue samples include CT scans, endoscopy, and ultrasound. CT scans will expose study participants to controlled amounts of limited radiation. The total dose of radiation from these tests is not anticipated to cause any adverse effects and is recorded for future review. The estimated radiation dose from a CT biopsy procedure is 258 mrem. For comparison, the average person in the United States receives a radiation exposure of 300 mrem per year from natural background source. There is also a risk of an allergic reaction to the intravenous contrast dye used during CT imaging, as well as a risk of experiencing feelings of anxiety or claustrophobia while undergoing a CT scan. Upper or lower endoscopy carries small risks of bleeding, infection, or perforation of the GI tract. There are no anticipated risks with the use of ultrasound.

In order to minimize these risks, participants will be queried, as per standard institutional practice, regarding their history of reactions to intravenous contrast dye. If a participant has had such a reaction, s/he will be pre-medicated, or dye will not be used, as per standard institutional practice. If a patient has previously experienced anxiety or claustrophobia while undergoing a CT scan, s/he will be encouraged to discuss this with the primary oncologist. For endoscopy, adequate preparation will be confirmed (e.g. fasting for 24 hours for upper endoscopy and removal of dentures; bowel prep for colonoscopy). Anxiolytics may be prescribed by the patient's primary oncologist as indicated.

Patient Privacy

TPF personnel will provide coded tissue and blood samples collected under LCCC1108 to researchers conducting genetic analysis.

All medical information abstracted from patient medical records under LCCC1108 will be recorded and stored in a secure password protected database. Medical records collected under the CSC will be downloaded into the LCCC Data Warehouse (LDW) managed by the LCCC Bioinformatics and Genomics Core.

As is standard with all trials that incorporate germ-line studies, we will inform patients of any implications for family members, and the risks associated with such studies, including those related to insurability and employment, and the limitations of laws designed to protect against such risks. Specific information

about heritable conditions will be communicated to participants by a trained genetic counselor.

4.4 Medical Records Abstraction

Medical records will be abstracted via the CSC, or by the LCCC1108 Study Coordinator for details on the following: cancer diagnosis, cancer stage, surgical and medical management, any treatment decisions made in response to the results communicated to the physician, PFS after the most recent therapy prior to UNCseq™ results on which the patient has just experienced progression, PFS after the first treatment post UNCseq™ results, PFS after any targeted treatment prescribed based on UNCseq™ results, and survival. Responses post treatment may also be documented. For eligible patients who are previously untreated at the time of 1108 enrollment and for whom no clear standard of care exists (e.g., metastatic or locally recurrent adenoid cystic carcinoma of the salivary glands), PFS prior to UNCseq™ results will be estimated from published data on the most commonly used regimens for the specific tumor. For patients whose prior treatment was therapy specifically targeted against their tumor (e.g., trastuzumab for HER2+ metastatic breast cancer), PFS prior to UNCseq™ results will be PFS post first-line chemotherapy in target refractory disease. In such cases, historical comparator rates will be determined a priori by members of the CCGR.

5.0 ADVERSE EVENTS

5.1 Definitions

5.1.1 Adverse Event

An adverse event or adverse experience (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

In this study, toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 (if appropriate), available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

5.1.2 Unexpected Adverse Event

An unexpected adverse event is an adverse event that is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

5.1.3 Serious Adverse Event (SAE)

An SAE is any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant disability/incapacity;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event that must be reported as an important medical event.

*Hospitalization for anticipated or standard of care specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor or recurrent disease, or elective surgery, will not be considered serious adverse events.

5.1.4 Unanticipated Problems

As defined by UNC's IRB, unanticipated problems involving risks to study subjects refers to any incident, experience, or outcome that:

- Is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Is related or possibly related to a subject's participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

5.2 Reporting

Any unanticipated problem that occurs during the conduct of this study and that meets **at least** the first two criteria listed in 5.1.4 must be reported to the UNC IRB using the IRB's web-based reporting system. If any SAE occurs in a patient undergoing a biopsy for research purposes only, the information should be recorded in the eCRF for that patient. Please include a full description of the event, its severity or toxicity grade, the relationship to the procedure, and the treatment, outcome and sequelae of the event.

6.0 STATISTICAL CONSIDERATIONS

6.1 Sample Size and Accrual

One primary objective of this study is to estimate the proportion of patients enrolled in LCCC1108 who have undergone successful sequencing and have a reportable genetic variant identified via UNCseq™. There is not a fixed sample size for this study. The number of specimens collected under this protocol will be reviewed on an annual basis and assessed in the context of ongoing and planned research. Based on this, a decision will be made, after discussion each year at the continuing review of the protocol, as to whether to continue this study. Based on enrollment into IRB # 01-1283 (LCCC0121: Molecular Analysis of Aerodigestive Cancers), we anticipate enrolling up to 5000 subjects into this study. The table below provides precision estimates for the proportion of patients with a reportable genetic variant for various sample sizes. As an example, with a sample size of 1000 patients, the maximum width of an exact 95% confidence interval (CI) will be 0.063. In other words, we will be able to estimate the proportion of patients with a reportable genetic variant within 3.1% if there are 1000 patients available who have undergone successful sequencing.

Total Successfully Sequenced Sample Size	Maximum Width of Exact 95% CI	Maximum Half Width of Exact 95% CI
500	0.089	0.045
750	0.073	0.036
1000	0.063	0.031
1250	0.056	0.028
1500	0.051	0.026
1750	0.047	0.024
2000	0.044	0.022
2250	0.042	0.021
2500	0.040	0.020
2750	0.038	0.019
3000	0.036	0.018
3250	0.035	0.017
3500	0.033	0.017
3750	0.032	0.016
4000	0.031	0.016

An additional primary objective of this study is to estimate PFS in cancer patients with active disease with a reportable genetic variant and those without a reportable genetic variant.

6.2 Data Analysis Plans

Patients will be evaluable for the primary objective of estimating the proportion of patients with a reportable genetic variant if they undergo successful sequencing via UNCseq™. A patient will be considered to have had successful sequencing as long as they fall into one of the following 4 groups:

1. Patients who had variants reported
2. Patients who had no variants reported
3. Patients who died before study results could be confirmed and so confirmation was halted
4. Patients whose only variants reported were confirmed by a standard of care assay that had been run in the clinical setting.

Patients included in group 1 (non-standard of care variant discovered via UNCseq™ and confirmed after UNCseq™ in a CLIA certified laboratory (or provided by a waiver from the IRB)) will be considered reportable for the purposes of this primary objective. The proportion of patients with a reported genetic variant will be estimated and reported along with an exact 95% confidence interval.

Only active cancer patients who have undergone successful sequencing will be evaluable for the other primary objective of estimatingPFS -in patients who have a reportable genetic variant and those who do not. Active patient status is determined at the time of MTB, as described in section 1.4.3. The Kaplan-Meier method will be used to estimate PFS and the 2 year PFS will be reported with its 95% confidence intervals using the log-log method.

7.0 STUDY MANAGEMENT

7.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the participant will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this

essential information has been provided to the participant and the investigator is assured that h/she understands the implications of participating in the study, the participant will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, s/he should sign and date the written informed consent agreement, preferably in the presence of the person who conducted the informed consent discussion, who will also sign and date the document.

7.2 Required Documentation

Before LCCC1108 can be initiated, the following documentation must be provided to LCCC1108 Study Coordinator.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study
- Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol
- CAP and CLIA Laboratory certification numbers and institution lab normal values

7.3 Registration Procedures

All patients must be registered with the LCCC 1108 Study Coordinator at the University of North Carolina before enrollment to study. For UNC patients, prior to registration, eligibility criteria must be confirmed with the UNC Study Coordinator. To register a patient, call 919-966-9259.

7.4 Data Management and Monitoring/Auditing

For tracking of specimens collected or accessed under LCCC1108, researchers will use password protected databases stored under secure conditions. TPF staff will track the number of vials procured (one specimen may be distributed into multiple freezing vials), the location of the vials in the freezer, and the utilization and distribution of any vial.

Abstracted clinical data will be collected into a secure, password protected database by Research Personnel from UNC LCCC.

As an investigator initiated study, this trial will also be audited by the Lineberger Cancer Center audit committee every six months.

7.5 Adherence to the Protocol

Except for an emergency in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

7.5.1 Emergency Modifications

UNC and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

7.5.2 Single Patient/Subject Exceptions

Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the UNC IRB.

7.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s)

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s)
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies

If a deviation or violation occurs, the guidelines below must be followed:

Protocol Deviations: UNC personnel will record the deviation in in a log, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

7.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written protocol amendment, and the amended consent form, if required, must be sent to UNC's IRB for approval prior to implementation.

7.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. For this protocol, study documents will be kept in accordance with institutional IRB guidelines.

7.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for ensuring that all the required data are collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator whose final signature will attest to the accuracy of the data.

8.0 REFERENCES

1. Peppercorn, J., et al., *Ethics of mandatory research biopsy for correlative end points within clinical trials in oncology.* J Clin Oncol, 2010. **28**(15): p. 2635-40.
2. Hirsch, F.R. and P.A. Bunn, Jr., *EGFR testing in lung cancer is ready for prime time.* Lancet Oncol, 2009. **10**(5): p. 432-3.
3. *Cetuximab (Erbitux®) Package Insert*. 2010, Imclone Systems Incorporated: Branchburg, NJ.
4. *Panitumumab (Vectibix®) Package Insert*. 2010; Available from: http://pi.amgen.com/united_states/vectibix/vectibix_pi.pdf.
5. Shaw, A.T., et al., *Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK.* J Clin Oncol, 2009. **27**(26): p. 4247-53.
6. Kwak EL, C.D., CLark J, et al., *Clinical activity observed in a phase I dose escalation trial of an oral c-MET and ALK inhibitor, PF-02341066.* J Clin Oncol, 2009. **27**(Suppl): p. 148s.
7. Kwak, E.L., et al., *Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer.* N Engl J Med, 2010. **363**(18): p. 1693-703.
8. Chabner, B.A., *Early accelerated approval for highly targeted cancer drugs.* N Engl J Med, 2011. **364**(12): p. 1087-9.
9. Bentley, D.R., *Whole-genome re-sequencing.* Curr Opin Genet Dev, 2006. **16**(6): p. 545-52.
10. Bookman, E.B., et al., *Reporting genetic results in research studies: summary and recommendations of an NHLBI working group.* Am J Med Genet A, 2006. **140**(10): p. 1033-40.
11. Robson, M.E., et al., *American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility.* J Clin Oncol, 2010. **28**(5): p. 893-901.
12. *National Comprehensive Cancer Network Guidelines: Genetic/Familial High-Risk Assessment: Breast and Ovarian* 2010 [cited 2011 March 8, 2011]; V1.2010:[
13. Ley, T.J., et al., *DNMT3A mutations in acute myeloid leukemia.* N Engl J Med, 2010. **363**(25): p. 2424-33.
14. *Massachusetts General Hospital Cancer Center News*. 2011 [cited 2011 March 5]; Available from: http://www.massgeneral.org/cancer/about/newsarticle.aspx?id=1590.
15. Christensen, K.D., et al., *Disclosing individual CDKN2A research results to melanoma survivors: Interest, impact, and demands on researchers.* Cancer Epidemiol Biomarkers Prev, 2011.
16. Von Hoff, D.D., et al., *Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers.* J Clin Oncol, 2010. **28**(33): p. 4877-83.
17. Janku, F., et al., *PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers.* PLoS One, 2011. **6**(7): p. e22769.
18. Janku, F., et al., *PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors.* Mol Cancer Ther, 2011. **10**(3): p. 558-65.

19. Janku, F., et al., *PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations.* J Clin Oncol, 2012. **30**(8): p. 777-82.
20. Hamilton, E.P., G.H. Lyman, and J. Peppercorn, *Availability of experimental therapy outside oncology randomized clinical trials in the United States.* J Clin Oncol, 2010. **28**(34): p. 5067-73.
21. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.
22. Cheson, B.D., et al., *Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol, 2003. **21**(24): p. 4642-9.
23. Cheson, B.D., et al., *Revised response criteria for malignant lymphoma.* J Clin Oncol, 2007. **25**(5): p. 579-86.
24. Cheson, B.D., *The International Harmonization Project for response criteria in lymphoma clinical trials.* Hematol Oncol Clin North Am, 2007. **21**(5): p. 841-54.
25. Cheson, B.D., et al., *Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group.* J Clin Oncol, 1999. **17**(4): p. 1244.
26. Durie, B.G., et al., *International uniform response criteria for multiple myeloma.* Leukemia, 2006. **20**(9): p. 1467-73.

9.0 APPENDICES

9.1 APPENDIX A

CHARTER

UNC COMMITTEE FOR THE COMMUNICATION OF GENETIC RESEARCH RESULTS

Objective of Committee

The UNC Committee for the Communication of Genetic Research Results (CCGR) has been established to create and periodically update the list of genetic tests that are appropriate for researchers to share with treating physicians and their patients within the context of LCCC1108. As an operating principle, researchers will not share genetic test results with treating physicians unless they can inform the care of a particular patient disease population.

Meetings:

The first meeting of CCGR will be scheduled to occur in Quarter 4 2011. The focus of the initial meetings will be to create the lists of reportable genetic tests (the "Master Gene Lists" as per LCCC1108). Follow-up meetings will occur quarterly for CCGR to review and update each list.

Master Lists

Disease Groups

To begin the process of generating the Master Gene Lists, the PI of LCCC1108 (Dr. H. Shelton Earp) will request that each cancer Disease Group (e.g., breast, head and neck, lung, etc.) provide the Chair of the CCGR (see list of members on page 4) a written summary of the standard of care (SOC) treatment/management by stage for diseases covered within their group. In generating these summaries, disease groups need to include the role of genetic alterations in this SOC management (e.g., to determine KRAS at diagnosis of mCRC, or to determine the status of HER2 at the time of breast cancer diagnosis, regardless of stage). This list of SOC genes will be compiled by tumor as List #1.

Through this process, disease groups will also identify when patients within their purview have run out of SOC treatment options, and would potentially be appropriate candidates for non-SOC treatment and/or for enrollment into clinical trials. They will also draft List #2, the list of non-SOC genetic alterations of potential clinical significance for review by the Pathology Committee.

Pathology Committee

The Pathology Committee (see list of members on page 4) will be responsible for reviewing the drafts of List 1 and List 2 prepared by the CCGR. As part of their review, the Pathology Committee must describe the validation status of the assay used to measure each genetic alteration. If a genetic alteration from the CCGR list #2 can be validated (either confirmed or refuted) in a CLIA environment, e.g., KRAS for use in the non-SOC setting, or verification of NextGen sequencing results by single nucleotide polymorphism (SNP) chip (or similar technology) in a CLIA environment, then the test will remain on List #2. If the genetic alteration from the CCGR list #2 cannot be validated in a CLIA research laboratory (e.g., IDH1), then it will be placed on List #3.

CCGR

CCGR will use reports, literature reviews, commercial or open-source products to evaluate the literature, and lists drafted by the Disease Groups and Pathology Committee to review and routinely update Master Gene Lists 1-2. List #1 will be categorized by tumor. For example, for any stage breast cancer, it is SOC to determine expression of HER2, PR or ER, while KRAS is SOC in CRC.

Treating physicians may consider using positive results from Lists 2-3 for participants who have run out of SOC options outside the context of a clinical trial. The point at which this occurs will vary for each participant according to tumor type/stage/clinical situation, as outlined earlier. As of the writing of Amendment #6, a List #4 is under consideration by the CCGR. This list would include genetic variants that predict sensitivity to non-targeted treatment (e.g. radiation, cytotoxics).

Individual Patient Reports

Researchers will prepare an Individual Participant Report from the genetic studies performed on a particular patient's tumor.

- If NextGen sequencing identifies a mutation in a SOC (list 1) test (e.g., the HER2/neu gene in a breast cancer patient), and the SOC test has not already been ordered by the treating physician, the Principal Co-Investigator of LCCC1108 will recommend that the treating physician order this test. In this case (which we anticipate will be rare), HER2 testing would be standard of care, and the participant and/or their insurance company would be charged for the SOC test (there will be no charge for the NextGen sequencing provided via the subject's participation in the study).
- For results from **List 2-3** the following will apply:
    - Prior to release to the treating physician, any positive genetic tests from List #2 will be analytically validated (whether confirmed or refuted) in a CLIA-certified laboratory. The participant (and/or their insurance

company) will not be charged for this test in a CLIA-certified laboratory as this will not be considered standard of care.

- After confirmation, results will not be released to the treating physician until the LCCC1108 molecular tumor board reviews and signs off. This tumor board provides additional oversight, ensuring compliance with the protocol, and appropriate application of results to individual patients. This board is comprised of representatives of the CCGR, the Pathology committee, key laboratory scientists involved in technical and bioinformatic aspects of UNCseq™, and clinical/molecular pathologists.
- Any positive results from List #3 must be reproduced via NextGen sequencing. These results may not be reported to the treating physician/participant until a waiver for the CLIA requirement is obtained from the UNC IRB. Therefore, results from List #3 (once they have been reproduced) must first be released to the LCCC1108 Study Coordinator. The Study Coordinator and the Principal Co-Investigator, Dr. Juneko Grilley-Olson, will work with the LCCC1108 Regulatory Associate to obtain a waiver from the IRB for the CLIA requirement. Once obtained, and the molecular tumor board has reviewed and signed off, results may be released to the treating physician/ participant with the following statement included "This is a research result from a non-validated test environment."

  In the case of List 3 results, any validation testing performed would not be charged to the participant and/or their insurance company.

Minutes

Minutes from each of the CCGR meetings will be recorded, and kept on a password-protected computer by the Committee Chair. A paper copy of all minutes will be maintained in the Regulatory files for this study.

Clinical Committee Members:

CHAIR: William Y. Kim, MD
Clinical Genetics: James P. Evans MD, PhD, Jonathan Berg MD, PhD
Developmental Therapeutics (Phase I): Claire Dees, MD
Breast Cancer: Lisa Carey, MD
Lung Cancer: Carrie Lee, MD
Melanoma: Carrie Lee, MD
Hematology: Kristy Richards, MD, PhD
Sarcoma: Hong Jin Kim, MD
GI Tumors: Autumn McRee, MD
GU Tumors: William Y. Kim, MD
Gynecologic Oncology: Victoria Bae-Jump, MD
Pediatric Oncology: Ian Davis, MD, PhD
Surgery: Hong Jin Kim, MD, David Ollila, MD
Radiation Oncology: Andrew Wang, MD
Ex-officio members of the Committee: H. Shelton Earp MD, Juneko Grilley-Olson MD

Pathology Committee Members:

CHAIR: David Allan Eberhard MD, PhD
Molecular Pathology/Genetics: Karen Weck-Taylor, MD
Pathology and Laboratory Medicine: Ryan Miller, MD, PhD; George Fedoriw, MD, Leigh Thorne MD, WilliamK. Funkhouser MD, PhD
Dermatology: Nancy Thomas, MD, PhD
Ex-officio members of the Committee: Norman E. Sharpless, MD