Section: Contact Information

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Section: Project Requirements and Description

Group: Requirements to submit AOI

A comprehensive review of previously published data has been completed. : **Yes**

The specific aims are clear and focused. : **Yes**

The investigator has appropriate experience and expertise to develop the concept proposal; if not, has identified a mentor or senior co-investigator. : **Yes**

The investigator agrees to develop an initial draft of the concept proposal within 6 weeks of approval of the AOI and to finalize the concept proposal within 6 months. : **Yes**

Project Title : **Immune Dysregulation and Premature Aging in Childhood Cancer Survivors: A Twin Study**

Planned research population (eligibility criteria) :
Childhood cancer survivors who are twins (both twins living).

Proposed specific aims :
SPECIFIC AIMS
Several decades ago, most pediatric leukemia or lymphoma patients (diagnosed under 21 years of age) died within 5 years. The development of a variety of new therapies has resulted in dramatic improvement with complete remission and cure in many of these patients. Accordingly, there is now a growing population of long-term pediatric and adolescent cancer survivors who are experiencing an increasing life span. However, the longer life span allows more time for mild to fatal late effects of chemo-, biologic and radiotherapy to manifest, including an elevated risk of higher of chronic fatigue, depression, Herpes zoster, early cardiovascular disease, pulmonary fibrosis, gastrointestinal reflux and second malignancies including myeloid leukemia and non-Hodgkin lymphomas. In fact for some pediatric cancers, such as Hodgkin lymphoma, the mortality from late effects is higher than that for the cancer itself. The contribution of premature imm une system and cellular aging due to the cancer and its therapy in young

patients has not been extensively studied. In addition, control comparisons are often inadequate. We (Cozen, Martinez-Maza and others) found evidence of altered inflammatory (higher) and Th1 (lower) cytokine levels associated with aging, Epstein-Barr viral copy number in PBMCs (higher), diversity of fecal flora (lower) and DNA methylation (greater) in the lymphoma survivor compared to their unaffected control twin.
Horvath recently developed a DNA methylation based biomarker of aging known as the "epigenetic clock", which can be used to measure the DNA methylation (DNAm) age of any human tissue, cell type, or fluid that contains DNA (with the exception of sperm). DNAm age of blood has been shown to predict all-cause mortality in later life, even after adjusting for known risk factors, which suggests that it relates to the biological aging process. Similarly, markers of physical and mental fitness are also found to be associated with the epigenetic clock (lower abilities associated with age acceleration). It is not yet known whether the epigenetic clock will lend itself for detecting accelerated aging effects in pediatric cancer survivors. In a small preliminary study, we have seen evidence for more rapid epigenetic aging in nine classical Hodgkin lymphoma survivors compared to their unaffected twins (Cozen, Wang and Horvath, preliminary observations).
We propose to build on our ongoing collaborations to test the hypothesis that long-term pediatric and adolescent cancer survivors have persistent immunological abnormalities, as well as premature biological aging that may be associated with adverse late outcomes. We will also test a novel hypothesis that microbial translocation (measured by serum immune markers) is associated with increased systemic inflammation, DNA aging and chronic fatigue in survivors. We will accomplish this by measuring a comprehensive panel of serum cytokines and chemokines and their receptors, in addition to novel markers of microbial translocation and DNA methylation age, in 212 pediatric leukemia and lymphoma survivors (average survival time = xx years) and their unaffected twins, who are participants in the Childhood Cancer Survivor Study (Armstrong, Bhatia). In a cross-sectional study, we will measure serum biomarkers, fecal microbiome and DNA methylation age in specimens from these participants, collected xx- xx years post-treatment. We will thoroughly assess quality of life, including life milestones and psychological and medical late effects, in survivors and correlate immunological and methylation biomarkers of aging in case samples collected prior to the current study to determine if alterations in these measures predict late effects.
The use of unaffected twins as controls mitigates the confounding effects of early life environment and genetic factors. To our knowledge, this is the first time a large-scale study of survivorship has been conducted in twins discordant for pediatric cancer.

The specific aims are to:
1. Define immunologic alterations associated with pediatric leukemia / lymphoma survivorship by quantifying systemic biomarkers of immune activation and inflammation: cytokines, chemokines, soluble cytokine receptors, markers of B cell activation, as well as biomarkers of microbial translocation from the gut

lumen into the circulation, in pediatric cancer survivors and their unaffected identical co-twins as controls;
2. Determine whether pediatric leukemia / lymphoma survivors have accelerated epigenetic aging compared to their unaffected twins, by measuring epigenetic age from DNA extracted from PBMC specimens;
3. Determine whether pediatric leukemia / lymphoma survivors have gut microbiome dysbiosis compared to their unaffected controls and whether it is correlated with serum biomarkers of immune activation and microbial translocation.
4. Determine the associations between inflammation/immune activation, premature aging, and microbial translocation measured in samples collected XX years ago and adverse late outcomes in pediatric leukemia / lymphoma survivors.

Will the project require non-CCSS funding to complete? : **Yes**

If yes, what would be the anticipated source(s) and timeline(s) for securing funding? : **NCI R01 submitted October 5**

Group: Does this project require contact of CCSS study subjects for:

Additional self-reported information : **Yes**

Biological samples : **Yes**

Medical record data : **Yes**

If yes to any of the above, please briefly describe. :
Note: all information and samples will be collected from the cancer survivor AND their unaffected (or affected) twin.

1- Questionnaire on health conditions, QOL, depression, BMI, physical activity, tobacco, alcohol, life milestones,
2-Samples: Blood, stool
3- Medical records- health conditions documented (See below)
4- Diet questionnaire

Group: What CCSS Working Group(s) would likely be involved? (Check all that apply)

Second Malignancy :

Chronic Disease : **Secondary**

Psychology / Neuropsychology : **Secondary**

Genetics : **Primary**

Cancer Control :

Epidemiology / Biostatistics :

Section: Outcomes or Correlative Factors

Late mortality :

Second Malignancy : **Primary**

Group: Health Behaviors

Tobacco : **Correlative Factors**

Alcohol : **Correlative Factors**
Physical activity : **Correlative Factors**
Medical screening :
Other :
If other, please specify :

Group: Psychosocial

Insurance : **Correlative Factors**
Marriage : **Correlative Factors**
Education : **Correlative Factors**
Employment : **Correlative Factors**
Other : **Primary**
If other, please specify : **Depression**

Group: Medical Conditions

Hearing/Vision/Speech :
Hormonal systems : **Primary**
Heart and vascular : **Primary**
Respiratory : **Primary**
Digestive : **Primary**
Surgical procedures :
Brain and nervous system : **Primary**
Other : **Primary**
If other, please specify : **Immunological including infections (ie infectious mononucleosis, Herpes zoster, TB), atopic conditions, autoimmune, diabetes, obesity, vaccinations**

Group: Medications

Describe medications :
for chronic conditions (ie metformin, statins) , antibiotic, antihistamine and antacid use, probiotics

Group: Psychologic/Quality of Life

BSI-18 : **Primary**
SF-36 :
CCSS-NCQ :
PTS :
PTG :
Other :
If other, please specify :

Group: Other

Pregnancy and offspring :
Family history : **Correlative Factors**

Chronic conditions (CTCAE v3) : **Primary**
Health status : **Correlative Factors**

Group: Demographic

Age : **Correlative Factors**
Race : **Correlative Factors**
Sex : **Correlative Factors**
Other :
If other, please specify :

Group: Cancer treatment

Chemotherapy : **Correlative Factors**
Radiation therapy : **Correlative Factors**
Surgery : **Correlative Factors**

Section: Anticipated Sources of Statistical Support

CCSS Statistical Center :
Local institutional statistician : **Yes**
If local, please provide the name(s) and contact information of the statistician(s) to be involved. :
David Conti, PHD
Acting Chief, Biostatics Division
Dept of Preventive Medicine
Keck School of Medicine of USC
dconti@usc.edu
Will this project utilize CCSS biologic samples? : **No**
If yes, which of the following? : **Other requiring collection of samples**
If other, please explain : **new sample collection of blood (separated into viably frozen T and B cells for methylation), serum for biomarkers and stool**

Section: Other General Comments

Other General Comments :
This is a study comparing childhood cancer survivors to their (mostly) unaffected twins. The shared genome and early childhood environment of twins makes this study design unusually powerful for determining true effects of cancer and its treatment. Therefore, although the main goals involve comparisons of biological data measuring immunological and cellular aging, it is also worth collecting the data to compare life milestones between the unaffected twin and the twin who developed cancer.