Grant Review Experience

- 09/2023 UK - CRUK's Discovery Research Committee, Expert Grant Review Panel
- 06/2023 Italy - Fondazione AIRC per la ricerca sul cancro ETS, Grant Reviewer
- 06/2023-2029 NIH, CSR, Cancer Prevention Study Section (CPSS), **standing member**
- 02/23/23 NIH, CSR, Cancer Prevention Study Section (CPSS), *ad hoc* reviewer
- 11/03/22 NIH, CSR, ZRG1 MOSS C(02) special emphasis panel (SEP), *ad hoc* reviewer
- 08/01/22 UK - Worldwide Cancer Research, Grant Reviewer
- 06/27/22 NIH, CSR, Cancer Prevention Study Section (CPSS), *ad hoc* reviewer
- 02/17/22 NIH, CSR, Nutrition and Metabolism in Health and Disease (NMHD), *ad hoc* reviewer
- 2022, 2023 Worldwide - Pfizer, Cachexia ASPIRE program, **standing member**

General Tips

- Short, basic sentences
- Short paragraphs ~10 lines
- Be mindful of white space on the page
- Avoid acronyms
- Avoid jargon
- Assume reviewers have not read your papers
- Assume you are speaking to a colleague in a similar department at another institution.

Reading your Submission

- Aims Page
- Significance & Innovation
- Approach

SPECIFIC AIMS

The incidence of colorectal cancer (CRC) is increasing among young and middle-aged adults around the world, but the reasons remain elusive. In the US, the rate of these cancers for those born in the 1980s and 1990s is now 2-4 times higher than for those born in the 1950s. These younger generations have been constantly exposed to low-cost, highly processed foods and drinks containing high-fructose corn syrup (HFCS), sucrose, and other fructose-containing sugars. Several, large, prospective cohort studies have linked fructose consumption to CRC risk and CRC-specific mortality but clinical studies of dietary influences on cancer are difficult to perform leading to inconsistent findings across all studies. In scrupulously controlled murine studies, we found that modest exposure to HFCS led to a substantial increase in tumor size and grade in the absence of obesity and metabolic syndrome (Goncalves *et al*., *Science*, 2019). However, the mechanism behind this growth remains unclear. A mechanistic link between dietary fructose and tumor growth would greatly support public health arguments for lower daily sugar intake and may unveil novel therapeutic targets.

Our long-term goal is to identify metabolic vulnerabilities of CRC and target them with small molecules. Our preliminary data demonstrate that fructose-exposed tumors have high levels of fructose 1-phosphate (F1P) and low activity levels of the glycolytic enzyme, pyruvate kinase M2 (PKM2). We show, for the first time, that F1P inhibits the activity of recombinant human PKM2 in a dose-dependent manner. Low PKM2 activity is known to increase cancer cell survival, particularly when oxygen is limited. We grew human CRC cells and intestinal organoids in low oxygen conditions and found that the addition of fructose to the media increases intracellular F1P, lowers PKM2 activity, and improves cell survival. This survival is blocked by the PKM2 activator, TEPP-46. Furthermore, we generated pilot data showing that TEPP-46 treatment blocks tumor growth in mice fed HFCS. Our central hypothesis is that fructose-derived F1P promotes CRC cell survival by inhibiting PKM2 and this vulnerability can be targeted with PKM2 activators. Since there are potent PKM2 activators in late-stage clinical development, the overall objectives of this proposal are to (1) establish the mechanistic links between fructose, PKM2, and tumor growth, and (2) define pyruvate kinase activators as a novel therapeutic modality for CRC.

Aim 1: To define PKM2 inhibition as the mediator of HFCS-induced tumor growth in mice.
Based upon our prior publications and preliminary data, we hypothesize that inhibition of PKM2 activity is essential for HFCS-induced tumor growth. Our approach will be to use established murine genetic models of CRC alone and in combination with conditional deletion of PKM2 in the colon to assess the effects of dietary HFCS on tumor growth. Complementary pharmacologic experiments will be performed in murine models using a PKM2 activator (TEPP-46) or placebo. Critical readouts include number and size of tumors. To ensure on-target activity, we will measure the abundance and activity of PKM2 in tumor lysates. We will also determine the effects on glucose and fructose metabolism using mass spectroscopy and heavy isotope tracers.

Aim 2: To define the mechanistic linkage between fructose exposure and cancer cell survival.
Our preliminary data show that fructose-exposed tumors have high levels of F1P and low activity levels of PKM2. When inhibited, PKM2 is known to stimulate HIF-1 transactivation of genes that promote cell survival. We hypothesize that fructose-derived F1P inhibits PKM2 and leads to expression of a hypoxia-defense program that promotes cell survival in oxygen-limiting conditions. Our approach will be to use established, human, CRC cell lines and patient-derived organoids to assess the impact of fructose exposure on cell survival under ambient and low oxygen conditions. The activity of PKM2 and ketohexokinase, the enzyme that produces F1P from fructose, will be increased and reduced using genetic and pharmacologic means. Critical readouts will include cell number and expression of hypoxia-defense genes and proteins. We will also determine the effects on glucose and fructose metabolism using mass spectroscopy and heavy isotope tracers.

Aim 3: To define F1P as a direct inhibitor of PKM2 and cancer-associated variants.
Our preliminary data shows that F1P inhibits the activity of recombinant, human, PKM2, however the mechanism of inhibition is unknown. We hypothesize that F1P is a direct inhibitor of PKM2 and cancer-associated variants. We will perform biochemical and structural studies using recombinant PKM2 to define the effects of F1P on the enzyme's kinetic parameters and elucidate the structural basis of their interaction. These studies will be repeated using naturally occurring PKM2 mutants found in human cancer to determine the contribution of these mutations to PKM2 structure and function. These experiments will reveal the molecular mechanisms of how F1P binds to PKM2 and alters the oligomeric state, which are important for a basic understanding of cell metabolism and future targeted drug design.

Together, these aims will: (1) define the fructose/F1P/PKM2 axis as a metabolic vulnerability of CRC, (2) provide pre-clinical evidence for PKM2 activators as a novel therapeutic modality to combat CRC, and (3) change our fundamental understanding of how fructose alters tumor cell metabolism.

Disease background
Significance
Gap in knowledge

Prior Publications
Preliminary Data
Hypothesis
Objectives

Aims:
Hypothesis
Approach:

- Models
- Methods
- Outcomes
- Rigor
- Technical innovation

Not too much detail
Not too little detail

More Significance

SIGNIFICANCE

Endometrial cancer (EC) is the most common gynecologic malignancy and the second leading cause of death from gynecologic cancer, causing an estimated 12,940 deaths per year in the United States and 97,370 deaths per year worldwide.[1,2] The incidence and death rate for EC continue to rise, in part, due to the obesity epidemic. Obesity is one of the strongest risk factors for EC and accounts for about 50% of cases in Europe and the US.[3] Obese women have a 3-fold increased risk of developing EC, and women with obesity and EC have an up to 6.25-fold higher risk of death compared to women of normal weight with this cancer.[3,4] Obesity induces a variety of systemic changes that favor tumor initiation and progression.[5] Some of these factors, such as hyperinsulinemia, have been directly implicated in the pathogenesis of EC, and may underlie the strong association of obesity with this cancer type.[6] **There is an urgent need for new therapies that target obesity-related factors to stop the progression of EC.**

The rigor of the prior work has strongly implicated insulin in the development of EC. Insulin and insulin-like growth factor-1 (IGF-1) stimulate the growth of the endometrial mucosa.[7,8] These hormones activate PI3K, a lipid kinase that directly phosphorylates several intracellular targets (e.g. AKT) that drive proliferation and growth.[9] PI3K activation is sufficient for the initiation of carcinoma in the native adult uterine epithelia,[10] and more than 90% of human endometrial tumors have alterations in the PI3K signaling pathway, most often activating mutations in *PIK3CA* (the gene encoding the active subunit of PI3K) or loss of function mutations in *PTEN* (the gene encoding a phosphatase that directly opposes PI3K).[11,12] Epidemiologic studies have identified significant associations among EC risk and clinical biomarkers of hyperinsulinemia such as high fasting insulin, c-peptide (a marker of insulin production), fasting glucose (a marker of insulin resistance), hemoglobin A1C (a marker of chronic hyperglycemia), and dietary glycemic load (a marker of dietary carbohydrate content).[11] **Despite the wealth of support for insulin and the PI3K pathway as drivers of EC development, studies of PI3K inhibitors have shown minimal efficacy in this setting with overall response rates (ORR) less than 10%.**[13–16] It is imperative that we understand the underlying mechanisms limiting their efficacy and explore new therapeutic approaches. This proposal will fill this critical knowledge gap.

Diet is the major source of nutrients that support tumor growth, yet the use of diet as a therapy is underutilized in the treatment of cancer. EC is the only tumor type where dietary carbohydrate (*i.e.*, glycemic load) is accepted as a risk factor for tumor development by the WCRF/AICR, international authorities on modifiable factors that associate with cancer.[17] Diets with 'very low' carbohydrates (VLCDs) reduce body weight and insulin levels in women with EC.[18,19] Yet, no studies have evaluated whether VLCDs can impact tumor progression, nutrient-signaling, growth pathways, or modulate the response to therapy. **This proposal will provide robust clinical evidence to support a VLCD as a key component of anti-cancer therapy. Recognizing the feasibility concerns with scaling the support required to allow for compliance with VLCDs, pharmaceutical alternatives (e.g., sodium glucose co-transporter inhibitor [SGLT2i]) are also proposed.**

Our group recently discovered that VLCDs and the SGLT2i class of anti-diabetic medication can improve the therapeutic response to PI3K inhibitors in various mouse tumor models.[20] SGLT2i's divert glucose into the urine without the need for insulin, rendering these drugs as potentially clinically highly viable partners in combination with PI3K inhibitors. In our preliminary data, we show that these combination therapies lower insulin and enhance the efficacy of PI3K inhibitors against EC organoids implanted into mice. **Since potent PI3K inhibitors and SGLT2i's are already available, this proposal will support the rapid implementation of novel combination therapies for EC (Table 1).**

Table 1. Anticipated Clinical Deliverables

Specific Aim	Major Clinical Deliverable
Aim 1. To examine the effects of insulin on EC signaling, growth, and the response to PI3K inhibition.	Pharmacodynamic evidence that the VLCD and VLCD+PI3K inhibitor combination therapy is reducing tumor nutrient-signaling and enhancing tumor cell apoptosis.
Aim 2. To identify novel strategies that lower insulin and enhance the apoptotic response to PI3K inhibition.	Identification of novel combination therapies including diazoxide+PI3K inhibitor and SGLT2i+PI3K inhibitor.

Furthermore, we will utilize multivariate modeling to unveil specific host factors and tumor molecular markers that predict a positive therapeutic response from these combinations.

- Does this study address an important problem or a critical barrier to progress in the field?
- If the aims of the application are achieved, how will scientific knowledge technical capability, and/or clinical practice be improved?
- What will be the effect of these studies on the concepts methods, technologies, treatments, services, or preventative interventions that drive this field?

Use the "set-up & deliver" approach

Make the Reviewers life easy by bolding statements that can be used in support of significance.

Innovation

Conceptual

Technical

Programmatic

Tips on Approach

- There is a gap in knowledge or controversy in the field
- Preliminary data is generated and reveals an interesting finding
- A hypothesis is generated
- Rigorous experiments are performed
- Results are obtained
- Conclusions are made

- Describe the model systems (SABV), sample size, primary outcome, statistical analysis plan, anticipated results, potential pitfalls and alternative strategies.
- Is it realistic to assume that you can do these experiments based on prior publications or preliminary data? If not, then find a collaborator.

NCI Transition Career Development Workshop: *What Reviewers Look for in your RPG Application*

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Who am I?

- Radiation Oncology Physician Scientist
- Clinical practice=central nervous system tumors
- Research focus=links between altered metabolism, DNA damage response and treatment resistance in brain cancers
- Grant Recipient (NCI, NINDS, Philanthropic Foundations)
- Grant Reviewer (RTB, SPORE Ad Hoc, NCI-J/Career Development standing)

What do I look for in a grant?

- Question 1: Should this work be performed?
  - Is it an important question? Will the discoveries resulting from this work have some chance (sooner or later) of helping the world?
- Question 2: If the money goes to this research team, will the work be completed?
  - Is it the right team of investigators with the right expertise/resources?
  - Is the experimental design correct? Are the model systems appropriate? Etc. etc.

General Advice (1)

- Know your audience
  - You are mostly writing for your 3 primary reviewers
- Write a compelling story
  - Don't assume that your reviewers will have read your published works or know relevant background information: the grant should stand alone as a story
  - Take liberties with formatting/where to put preliminary data if it is the service of making the grant easier to read and understand
- Your aims page is the most important page in the grant and should get your reviewers excited

General Advice (2)

- Grant writing is planning. When it's well done, it can elevate your science and sharpen your ideas even if it's not funded
- Plans are worthless but planning is essential
  - A good budget/justification lets the reviewers know you've thought through all the steps of your research
  - A well-written pitfalls/alternative approaches lets reviewers know that the money won't be wasted if the first idea is wrong
- If you haven't published with a collaborator before and there is no money moving, then I worry about that collaborator's part getting done