Request for Applications (RFA) on Novel Biomarkers for the Partnership for Accelerating Cancer Therapies

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C. ISSUED BY:	**D. ADDRESS OFFERS TO:**	
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IMPORTANT: **F.** To be considered for award, Offers must be received at the location specified in Block E.2. above by 11:59PM EST March 31, 2021. Offers must be clearly identified with the solicitation number provided in Block A above.		

The Foundation for the National Institutes of Health, a non-profit, 501(c) (3) charitable organization that supports the NIH in its mission to improve health by forming and facilitating public-private partnerships for biomedical research, is issuing a Request For Applications (RFA) for novel biomarkers to support the Partnership for Accelerating Cancer Therapies (PACT). PACT seeks to provide a systematic approach to immune and related oncology biomarker investigation in clinical trials by supporting development of standardized biomarkers and assays. PACT is executed by FNIH as a public-private partnership involving the National Cancer Institute (NCI), the U.S. Food and Drug Administration (FDA), multiple pharmaceutical companies, non-profits and patient advocates.

Purpose

This Request for Applications is associated with the Partnership for Accelerating Cancer Therapies (PACT), a project associated with the Beau Biden Cancer Moonshot ™ Initiative that is intended to accelerate cancer research. The overall goal for this Request for Applications (RFA) is to develop and validate novel biomarkers that accurately predict response to immuno-oncology interventions, including combination therapies, monotherapies, vaccines, and other novel immunotherapy treatments.

Background

Recent advances in cancer treatment have offered the prospect of greatly enhanced outcomes, improved duration of survival, and cure for some patients. Much of the recent success has been driven by the development of new immuno-oncology (IO) agents, leading to an explosion of translational research as well as investment in the field. To date, however, the improvements in outcomes and cure generated by the monotherapies of these agents are possible only for a minority of patients, and emerging data demonstrate that the greatest impact on cancer treatment will be achieved by combinations of multiple IO

agents or of IO and non-IO agents. The identification of successful combinations is a complex process due to the number of possible combinations, tumor heterogeneity, and the need for new translational biomarkers and fit-for-purpose assays to identify patients for relevant combination therapies. These challenges are further compounded by the novelty and intensely competitive nature of the IO field, which has encouraged fragmented and at times duplicative research approaches.

To solve these challenges, a systematic cross-sector effort is required to identify and develop robust, standardized biomarkers and related clinical data that support the selection and testing of promising therapeutic combinations. To enable achievement of these goals, the National Institutes of Health (NIH) and multiple pharmaceutical companies have formed a 5-year, ~$220 million precompetitive public-private research collaboration called the Partnership for Accelerating Cancer Therapies (PACT).

PACT facilitates robust, systematic, and uniformly conducted clinical testing of biomarkers that enable researchers and clinicians to better understand the mechanisms of response and resistance to treatment strategies. PACT will provide a systematic approach to immune and related oncology biomarker investigation in clinical trials by providing standardized and harmonized basic and exploratory biomarker assays, which can be utilized within the PACT programs and across the research community. The harmonization of these assays allows for (a) consistent generation of data, (b) access to uniform and harmonized assays to support data reproducibility, (c) comparability of data across trials, and (d) discovery/validation of new biomarkers for combination immunotherapies and related combinations.

This RFA has been released to drive the discovery and validation of new biomarkers in (d). To this point, PACT has selected three primary areas on which to focus this RFA:

1) T Cell Receptor (TCR) Repertoire and Epitope Prediction
2) Epigenetic Biomarkers
3) Novel TME Imaging Techniques and Digital Spatial Analysis

Biomarkers developed from this RFA funding in these key areas will be expected to take into account already ongoing biomarker assay development work that has been undertaken as part of the PACT-CIMAC-CIDC Network efforts. These efforts, which have already been funded by the National Cancer Institute (NCI) and PACT, have established a network of Cancer Immune Monitoring and Analysis Centers (CIMACs) and a Cancer Immunologic Data Commons (CIDC) to provide consistent, harmonized biomarker assays and data repository for NCI's extramural clinical trial networks. The NCI made awards to 4 academic research institutions: The University of Texas MD Anderson Cancer Center (PIs: Ignacio Wistuba, Cara Haymaker, Gheath Al-Atrash), Icahn School of Medicine at Mount Sinai (PI: Sacha Gnjatic), Stanford University (PIs: Holden Maecker, Sean Bendall), and Dana-Farber Cancer Institute (PIs: Catherine Wu and F. Stephen Hodi).

To this end, the PACT team is providing here the current list of assays that have been selected by the CIMACs for standardization, harmonization, and development (**Figure 1**). Those assays indicated as Tier 1 assays are those that have been standardized and harmonized across centers and are the priority for incorporation into trials. The data from these assays is also the primary focus for initial incorporation into the CIDC. The PACT team feels that this background understanding is essential, so the applicant can understand the level of novelty necessary to be considered for this award.

Category	Tier 1	Tiers 2-3
Tissue Imaging	Multiplex immunohistochemistry and immunofluorescence (Multiplex IHC/IF)	Conventional immunohistochemistry
		Multiplexed Ion-Beam Imaging (MIBI)
Cell Profiling	Mass Cytometry (CyTOF)	High-dimensional flow cytometry
		ELISpot
Sequencing	Whole Exome Sequencing (WES)	Assay for Transposase-Accessible Chromatin (ATAC-seq)
	RNA-Seq	TCR/BCR Clonality
	Nanostring	HLA-Seq; Epitope Prediction
		ISH DNA/RNA
		Neoantigen Prediction
		Circulating Tumor DNA
		HTG-EdgeSeq (gene expression)
		Microbiome (16S Deep Sequencing)
		Single-cell RNA-sequencing (scRNA-seq)
		Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq)
Cytokines/Serum Analytes	O-link Serum Cytokine Analysis	Luminex
		ELISA/Grand Serology
		MesoScale Discovery

Tier 1 = broadly recommended for all trials

Tiers 2-3 = exploratory, usage depends on the trial

Figure 1. Current CIMAC and PACT-selected Tier 1 and Tier 2 assays

The PACT team drafting this RFA has considered this ongoing work in selection of our topic areas of focus, which are further delineated below; therefore, the team would ask that applicants consider how their proposed assays could enhance this ongoing work or develop novel assays and biomarkers not already in the standardization and harmonization process.

Topic Area 1: T Cell Receptor (TCR) Repertoire and Epitope Prediction

Immunotherapy has achieved great success in treating cancer patients, but it derives clinical benefits only a subset of patients. Although promising response biomarkers have been identified, to date no single biomarker provides satisfactory predicting power. Thus, an integrated analysis of multiple data types is highly needed.

Several predictive biomarkers that show promising results in response to checkpoint inhibitor therapy have been reported (*Havel JJ et al., 2019*). Specifically, tumor mutation burden (TMB), PD-L1 expression, T cell-inflamed tumor microenvironment (TME), and recently microbiome composition and changes of T cell receptor (TCR) repertoire clonality have been shown to be some of the promising predictive biomarkers. Notably, TCR-Seq assay has also been listed under Tier 2 panel as one of the PACT CIMAC-CIDC Network assays.

TCR repertoire sequencing has been employed as a surrogate biomarker for detecting a minimal residual disease (MRD) in blood cancers and recently for predicting clinical outcomes and response to immunotherapy (*Wu TD et al., 2020; Cowell LG, 2020*). Significant efforts to utilize TCR sequencing platform to identify mechanism of action of therapeutic agents are ongoing. These efforts also expand into

identifying specific target antigen or neoantigen by deploying immunoinformatic approaches (*Glanville J. et al., 2017; Fischer DS et al., 2020; Zvyagin IV et al., 2020*).

Typically, neoantigen screening is a combined effort between mutation detection by whole exome sequencing (WES)/RNA sequencing (RNA-seq) and *in silico* neoepitope prediction by bioinformatic algorithms. Ultimately, these hypothetical neoantigens need to be further validated by immuno-peptidomics using the LC-MS platform. This process is quite long and complex and it has a limited utility in practice specifically due to limited peptide detection from small quantity of tissue samples. Moreover, the differences between *in silico* prediction and experimental data add to this complexity.

To address this gap, we are proposing to generate novel bioinformatic approaches to identify neoepitopes or tumor-associated antigen epitopes based on modeling of historical large sets of data on TCR sequence and its putative epitope information. Here, the integration of strong bioinformatics components that include comprehensive coverage of TCR repertoire and computation biology capacity with testable algorithms are key factors for these approaches to be successful. In addition, these approaches need to have the potential to be deployed in clinical settings with statistical significance.

Topic Area 2: Epigenetic Biomarkers

The abnormal epigenomic landscape is one of the hallmarks of tumor initiation and progression (*Esteller, 2008; Tsai and Baylin, 2011*). Epigenetic modifications such as DNA methylation and post-translational histone modifications are alterations that lead to the aberrant expression of tumor-associated genes that drive cellular malignant transformation and cancer progression. In particular, aberrant modifications of 5-methylcytosine (5mC) profiles can promote uncontrolled cell proliferation and survival, as well as supporting tumor growth and silencing of tumor-suppressor (TF) genes through promoter hypermethylation (*Bird, 2007*). In addition, post-translational histone modifications impact gene expression by modulating chromatin conformation and accessibility. Similar to histone acetylation, histone modifications are associated with gene transcription activation. Depending on the methylation marks, histone methylation can lead to either translation activation (i.e. H3K4me3) or repression (i.e. H3K9me3, H3K27me3).

Many of the epigenetic regulators are expressed in tumor cells and different immune cell types. Thus, these epigenetic regulators control the function of immune cells and tumor cell properties. For example, epigenetic regulators affect the expression of genes in the antigen presentation machinery such as major histocompatibility complex class I (MHCI) (*Burr et al., 2019*). Moreover, epigenetic reprograming in cancer cells has an impact on immune cells in the tumor microenvironment (TME). Data show that tumors co-opt certain epigenetic pathways, silence T_H1-type chemokine expression and repress T-cell tumor homing, which functions as a novel immune-evasion mechanism (*Peng et al., 2015*). When expressed in immune cells, epigenetic regulators may repress genes that lead to differentiation of T-helper cell subsets, silence CD8 effector differentiation and function, increase the suppressive activity of T-regs, and inhibit NK cell activation (*Gray et al., 2017; Schientinger et al., 2016; Ghoneim et al., 2017*). Therefore, targeting epigenetic elements that promote tumor progression and inhibit immune cell activity can enhance antitumor immunity by reshaping the TME.

Current epigenetic drugs that target aberrant epigenetic dysregulations are being evaluated through clinical studies with the goal to prevent resistance to chemo/ other targeted therapies, prevent lineage shift, make cells responsive to immunotherapies and improve therapeutic specificity (*Cheng et al., 2019*). At present, there are hundreds of ongoing single-agent or combinatorial trials where epigenetic drugs are combined with standard of care (SoC) therapies like chemotherapy and immunotherapies. Although these drugs as monotherapies do not provide clinical benefit, their synergy with immune checkpoint blockade (ICB) agents is expected that can be supported by execution of translational studies for deriving proof of concept. At the end of 2019, 48 trials were documented where ICB drugs were combined with epigenetic

inhibitors like EZH2Ii, HDACi, DNMTi across many indications such as melanoma, non-small cell lung, urothelial carcinoma and others. Generating translational data and identify epigenetic biomarkers of response from these trials is highly desirable, as they will aid in identifying patient subsets that may benefit from ICB + Epigenetic inhibition and thus will aid in patient enrichment strategies for subsequent studies. The data from these translational investigations will allow for future integrated comparisons of samples through correlative approaches and may further provide rationale for combinatorial strategies.

The scientific target for responses to this topic area would be to propose the identification of novel epigenetic biomarkers through testing or assays in animal models or human cancer samples. The goal of these proposals would be to understand the impact of epigenetic regulators in inducing changes in the tumor and the TME. Also, studies that propose approaches on how to use epigenetic drugs to generate vulnerabilities in cancer and immune cells that lead "priming" of tumors to ICB are highly desirable.

Topic Area 3: Novel TME Imaging Techniques and Digital Spatial Analysis

The tumor microenvironment (TME) is a complex and heterogeneous matrix of tumor, stroma and a diverse immune infiltrate. Immune suppression in the TME hampers productive antitumor immunity leading to resistance to SoC therapies and ICB. The landscape of TME and its impact on tumor progression across indications has led to systematic efforts to better define genomic and transcriptomic signatures that are aberrant in cancer and govern interactions between tumor and the TME (*Thorsson et al., 2018; Hoadley et al., 2018; Campbell et al., 2018*). However, these efforts lack the spatial context of expression of nucleic acid, protein, cellular, and/or morphometric markers and cells that express them in the TME. For this, recent advanced analytical capabilities in the field of digital pathology have proven useful for biomarker exploration and discovery.

In the recent years, novel technologies like Spatial Transcriptomics and multiplex fluorescence assays (Codex, CycIF, Akoya-mIF, MIBI) have aided in interrogating the spatial location of immune and other stromal cells in the TME coupled with knowledge on their functional cell states and identity based on proteomics and transcriptomic profiles. For example, *Schurch et al., 2020*, interrogated how the spatial organization of tumor and immune cell populations within the TME give rise to distinct cellular neighborhoods (CN) that are linked to clinical outcomes using FFPE-Codex multiplexed tissue imaging of 56 markers in 140 tissues from 35 patients with colorectal carcinomas (CRC). Their studies showed that in low- versus high-risk patient, there is an altered organization of the tumor and the TME, where local enrichment of PD-1+CD4+ T cells within a CN of granulocytes correlates with survival in high-risk patients. All together, these studies demonstrate that such novel techniques can capture the spatial organization and functional status of various immune cell types. Furthermore, these findings help elucidate the concerted efforts of multiple immune cell types that help generate the complex biological response of anti-tumor immunity. This information will be critical to identify novel biomarkers as the field moves towards novel immunotherapy agents that target myeloid cells, NK cells, in addition to T cell targeted therapies into the clinic.

To further develop these prospective biomarkers, the goal of this topic area for proposals is to deploy novel technologies that combine functional and transcriptomic signatures with spatial information in cancer patient cohorts treated with IO +/- SoC (i.e.: chemotherapy, radiation therapy, hormone therapy, targeted therapy) and for which clinical annotation is available. The overarching goals aim to:

a. Interrogate the spatial organization and molecular characteristics of the "triad": tumor cells, stromal factors and immune cells. Identify pathways that govern immune phenotypes, landscapes, and stromal structures in the context of clinical response to IO +/- SoC

b. Identify novel predictive spatially defined biomarkers and propose a plan to assess prospectively (or in retrospective cohorts) their value in predicting clinical response to ICB +/- SoC monotherapy/combination clinical benefit.

Specific Research Objectives and Requirements

This RFA solicits applications for proposals to develop and analytically validate novel biomarkers that accurately predict response to immuno-oncology interventions, including combination therapies and monotherapies, with the goal of advancing cancer treatment and research. The overarching technical objective for responses to this RFA within the scientific topic areas above is to develop assays which satisfy the analytical validity definition as adopted by the Institute of Medicine. Standardization of the assay is critical, and the responses should outline the planned studies to be conducted to address analytical validity, such as evaluation of standardized reference materials with known characteristics along with cross-platform comparisons as recently outlined in a joint review by American Society of Clinical Oncology and College of American Pathologists (*Merker JD, 2018*). The ultimate eventual goal of the PACT effort is to assess the clinical validity and clinical utility of assays developed as part of this partnership. The proposed projects within the responses should be designed such that the assay developed could be incorporated into the clinical setting by the conclusion of the term of the award.

The conduct of clinical trials is outside of the scope of this current RFA, but applicants should take into consideration that the ultimate goal of the biomarkers developed will be clinical validation and eventually proof of clinical utility.

The topic areas of investigation targeted by this RFA are as follows:

1) For the topic area of T Cell Receptor (TCR) Repertoire and Epitope Prediction, a successful RFA would include:
    a. RFA plans for the following assessments
        i. Predicting tumor (neo)antigen and its binding specificity from recent TCR sequencing datasets for novel mechanism of response or resistance
        ii. Predicting TCR clonality for treatment response incorporating novel algorithms including tumor (neo)antigen specificity
    b. RFA characteristics
        i. Technology requirements on consistency, comprehensive coverage, and bioinformatics integration
        ii. Solid bioinformatics component and testable algorithm
        iii. Enough database and (clinical) sample size for Training, Validation, and Testing sets for statistical significance
        iv. Clinical correlations and feasibility in clinical settings

2) For the topic area of Epigenetic Biomarkers, a successful RFA would include plans for the following primary assessments:
    a. To identify biomarkers/ epigenetic mechanisms of primary or acquired resistance to IO single agent or IO combination therapies.
    b. To identify novel and more potent predictive epigenetic biomarkers to identify patients that benefit from ICB + Epi drugs
    c. To identify epigenetic- based modifications which can serve as non-invasive tools for monitoring response to ICB +/- Epi drugs

3) For the topic area of Novel TME Imaging Techniques and Digital Spatial Analysis, successful RFA characteristics would include the following:
    a. Sufficient sample size

b. Patients treated with ICB, ICB and SoC, or other IO therapeutics
c. Clinical outcomes from trial available
d. Pre-, On, and Post-treatment samples from each patient are ideal
e. Plan or process to follow up with candidate biomarkers within prospective study or a validation dataset
f. Experimental and clinical correlations

Award Information

I. Funds Available and Anticipated Number of Awards

The number of awards and the amount per award is contingent upon the submission of a sufficient number of meritorious applications and proper budget justification within the proposal.

II. Award Budget

Application budgets are limited to an amount up to $500,000 per year and need to reflect the actual needs of the development of the proposed biomarker/assay. Proper scientific and budget justification will need to be provided for evaluation. The committee reserves the right to award at a lower amount than requested.

III. Award Project Period

The scope of the proposed biomarker assay work should determine the award project period. The maximum project period is 2 years with the potential for a no-cost extension to complete the necessary analyses if the sample collection for the study lasts the duration of the original award.

Eligibility Information

Organizations eligible to apply are:

- Private or public sector
- US-based or international
- Able to comply with the necessary PACT IP, data sharing, and publication guidelines (Guidelines documents are available upon request).

Application and Submission Instructions

I. Submission Deliverables

Complete applications will include:

- Application write-up which should describe the information below, but more details can be provided in the application response template (Appendix 1):
  - biomarker assay(s) to be developed
  - rationale for why this biomarker would benefit the IO field, especially the advancement of patient selection for drug treatment
  - current state of the biomarker (i.e. – early stages, assay developed but need analytical validation, analytical validation completed but will need clinical validation, etc.)
  - personnel that will conduct the work
- Detailed budget that delineates (An example budget table can be found in Appendix 1)
  - Personnel
  - Reagents and materials

    - Equipment
    - Sample acquisition (if necessary)
    - Other requirements for work proposed
- Detailed budget justification
- Proposed project timeline
- Biosketches from the Principal Investigators (Should not exceed 3 pages)

II. Data, Publications and Intellectual Property

All applicants will be expected to comply with the PACT Policies and Guidelines that have already been established for the partnership. These are available upon request and will be attached to any award agreements for those projects selected for funding.

III. Page Limit

Please keep your responses under 15 pages in length (single spaced, font 11 pt) not including biosketches. Further section length suggestions are provided in Appendix 1.

IV. Award Reporting

For those applications selected for award, the Principal Investigators on the award should expect to submit progress updates for the project every 6 months in a format that will be described in the award agreements.

V. Additional Information Required

Please provide any existing IP or patent information relevant to the assay that may affect its use in the partnership, or the banking of any resulting data funded by this effort in a public controlled access database for use after initial publication of the findings. Further guidance available upon request.

VI. Submission Instructions

Send responses via e-mail to PACT@fnih.org with a copy to Jenny Peterson-Klaus, Senior Scientific Project Manager, Cancer Research Partnerships (jpeterson-klaus@fnih.org); and Dr. Stacey J. Adam, Director, Cancer Research Partnerships (sadam@fnih.org).

Key Dates

Application Due Date: March 31, 2021, 11:59 PM EST

Targeted Application Review Period: April 1, 2021 – May 1, 2021

Potential Oral Presentations from Finalists (*If Needed*):
Applicants will be informed after initial review of proposals whether they will need to provide and oral presentation with the ability for Q&A to the PACT RFA Review Team

Targeted Award Announcement: Q2 2021*
Applicants will be notified by email of the outcome of the RFA.

**If no adequate submissions are received in this timeline, the FNIH reserves the right to extend the target deadline.*

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