The Human Tumor Atlas Network (HTAN)

Frequently Asked Questions (FAQs) about HTAN and the Newly Published Notices of Funding Opportunity (NOFOs)

We appreciate your interest in the Human Tumor Atlas Network (HTAN) and hope that you and your team will choose to submit an application. To maximize your chances of success, we would like to provide some guidance that may be helpful. For additional clarification of these or other issues, we encourage you to contact the appropriate HTAN team via email (contacts are listed in the Requests for Applications [RFAs]).

The three recently published HTAN NOFOs are:

- RFA-CA-23-039 - Human Tumor Atlas (HTA) Research Centers (U01 Clinical Trial Not Allowed)
- RFA-CA-23-040 - Pre-Cancer Atlas (PCA) Research Centers (U01 Clinical Trial Not Allowed)
- RFA-CA-23-041 - Single Source: Human Tumor Atlas Network Data Coordinating Center (HTAN-DCC; U24 Clinical Trial Not Allowed)

An applicant must note that submission of duplicate or overlapping applications to both RFAs (RFA-CA-23-039 and RFA-CA-23-040) is not acceptable.

General Questions on HTAN

1. How is a human tumor atlas defined?

Within the HTAN, a human tumor atlas is generally defined as the multidimensional molecular, cellular, and morphological mapping of human cancers, complemented with critical spatial information (at the molecular, cellular, and/or tissue level) that facilitate visualization of the structure, composition, and multiscale interactions within the tumor ecosystem. Tumor atlases constructed within the HTAN should describe the dynamics of cancer, focusing on the transition from precancer to malignancy, from local invasion to distant metastasis, rise of locoregional and/or distant recurrence, and response to or development of resistance to treatment. Please see the above-mentioned RFAs for more information.

2. What are the overarching goals and scope of HTAN under the new NOFOs?

The overarching goal of the HTAN (HTA and PCA Research Centers) is to map tumor evolution using multimodal approaches, advanced multiplex technologies, and spatial image analysis (preferably 3D analysis) to capture the extensive interactions within precancerous lesions and advanced tumors and the surrounding ecosystems as a function of space and time. These comprehensive atlases will allow the development of new classifiers for risk prediction, biomarkers for early detection, identification of potential targets for preventive interceptions, enhancement of diagnostic and treatment strategies for advanced cancers, and generation of hypotheses and insights to facilitate future research on underlying biological mechanisms.

The HTAN will support two types of atlas-building initiatives, and brief information is provided below. Please peruse the HTA and PCA RFAs for additional details about atlas development.

> **HTA Research Centers (RFA-CA-23-039)** will construct atlases describing one or more transitions spanning the entire cancer continuum: precancer to locally invasive cancer, early

invasive cancer to more advanced stages and metastatic cancer, relapse/local recurrence, dynamic response to therapy, and development of therapeutic resistance. The atlas efforts must leverage and expand upon the current HTAN resources and infrastructure and focus on creating spatial atlases at single-cell resolution that are driven by specific topics in cancer biology across the continuum from cancer initiation to metastasis, development of relapse/recurrence, and treatment response and/or resistance. HTA Research Centers will use dissociative singlecell/nuclei or bulk sequencing or other molecular assays in a supporting role to the proposed spatial analysis.

PCA Research Centers (RFA-CA-23-040) will construct atlases characterizing precancerous lesions and their transitions to invasive cancers or regression or stabilization of the lesions. For cancer prevention, it is important to characterize at-risk tissues, including regions (tissue microenvironment) adjacent to the precancerous lesion. Where applicable, the interplay between somatic mutations and at-risk tissues and tissues associated with inflammation and other chronic conditions should be taken into consideration. Tissues associated with germline mutations provide opportunities for the joint study of germline and somatic variants in relation to disease initiation and progression. Proposed atlases should discuss such complexities in the histologically normal tissues and illustrate the relationships with molecular, cellular and tumor microenvironment features to define the progression from precancer to cancer.

3. What is the role of the HTAN Data Coordinating Center (DCC)?

The role of HTAN-DCC will be to collect, store, curate, and disseminate all data, metadata, experimental protocols and standard operating procedures, and analysis and visualization tools generated by the HTAN. Additionally, the DCC will lead the development and implementation of common data elements, data and metadata standards, clinical and epidemiological data requirements, and data processing pipelines. The DCC will also coordinate HTAN activities including in-person and virtual HTAN Steering Committee meetings, subcommittees, and working groups. Finally, the HTAN-DCC will promote collaboration and communication between HTAN investigators and the broader research community and coordinate Network outreach activities. The HTAN-DCC, HTAN investigators, and NCI staff will need to work closely together to accomplish the goals of the HTAN-DCC in a manner that will best benefit all HTAN investigators and the broader scientific community. Please see the DCC RFA for more details.

4. What is the relationship between HTAN and other similar atlas-construction programs?

There are several organized single-cell atlas programs funded by the NIH (HuBMAP, SenNet, BRAIN Initiative Cell Census Network, GUDMAP, LungMAP) as well as the international community (Human Cell Atlas, Chan Zuckerberg Initiative Seed Networks) that are focused on building molecularly resolved atlases of normal tissues. Additional efforts are systematically mapping specific non-malignant (NIH Kidney Precision Medicine Program, Helmsley Gut Atlas) or premalignant (Gray Foundation BRCA Team Science Project) disease states. Currently, the HTAN investigators actively participate in events, special interest groups, and/or working groups that promote collaboration and resource sharing across atlas efforts, and will be expected to continue to do so in the next phase of HTAN.

5. What consortia agreements are expected in the HTAN Program?

Awards funded under HTAN NOFOs are anticipated to involve activities conducted by multidisciplinary teams of investigators. HTAN awardees will form a consortium. In addition to completing the research goals outlined in their applications, successful applicants will be expected to work collaboratively with all

members of the HTAN, including the HTAN-DCC. The HTAN will encourage the initiation of new collaborative research projects across the entire network. New HTAN awardees joining under the 2023 HTAN NOFOs will be expected to sign and follow the HTAN-developed agreements, such as HTAN policies governing joint publications, material transfer agreements, and data sharing that were ratified during the initial HTAN phase.

All HTAN investigators will be required to attend the initial HTAN Kickoff meeting, as well as semi-annual HTAN investigator meetings and regular teleconferences with Network members and NIH Program Staff for the duration of the funding cycle. Further details of the responsibilities of the HTAN investigators, including those associated with the HTAN Steering Committee, can be found in Section VI. Award Administration Information under "Cooperative Agreement Terms and Conditions of Award" in each HTAN FOA.

6. What are the plans for data and resource sharing for this program?

The HTAN is a community resource generating program, intended to provide data and resources for use by the cancer research community. All HTAN Research Centers must ensure that data are deposited to the HTAN-DCC in accordance with HTAN and NIH/NCI policies, and that models, software, and other tools and resources developed as part of this Research Center are made publicly available according to HTAN policies and in accordance with the Beau Biden Cancer Moonshot Public Access and Data Sharing Policy and the NIH Data Management and Sharing Policy. Applicants must also state the degree to which the resulting processed data is interoperable and meets the qualifications for FAIR data sharing standards. Applicants should ensure their ability to follow HTAN data and resource sharing expectations before applying for the program.

7. What is the role of the HTAN Steering Committee?

Once HTAN awards have been made, the HTAN Steering Committee that is responsible for joint governance of HTAN activities will be constituted (see Section VI. Award Administration Information under "Cooperative Agreement Terms and Conditions of Award" in each HTAN NOFO). The HTAN Steering Committee will be responsible for:

- Identifying scientific and policy issues that need to be, or can benefit by being, addressed at the Network level and develop recommendations to NIH/NCI Program Officials for addressing such issues.
- Ensuring progress of the HTAN toward meeting the overall Network goals.
- Ensuring that all HTAN members are utilizing the resources developed by the HTAN-DCC.
- Discussing and prioritizing the collaborative projects to be supported by the restricted "HTAN Pilot and Trans-Network Projects" funds within each Research Center.
- Coordinating, organizing, and disseminating Network output to the broader cancer research community, potentially including the organization of coordinated publications and/or presentations.
- Ensuring that the Network takes advantage of existing NCI and NIH resources and programs.
- Establishing, as necessary, working groups, special interest groups, or subcommittees to accomplish the goals of the HTAN program.

8. Is funding based on a payline?

No. Funding decisions are based on scientific merit and programmatic needs, as defined in the NOFOs, and on the availability of funds.

9. What role does the NIH/NCI have in the HTAN?

The role of NCI staff in the HTAN is elaborated in each NOFO in the Cooperative Agreement Terms and Conditions of Award section. Briefly, NIH/NCI staff will have substantial programmatic involvement that is above and beyond the normal stewardship role in awards.

Questions specific to HTA RFA (RFA-CA-23-039)

Questions on RFA Scope and Purpose/Expectations

10. What is the main objective of the HTA RFA?

The objective of each HTA Research Center is the construction of no more than one tumor atlas that describes the molecular, cellular, and tissue-level spatial, functional, and/or temporal relationships of cellular and non-cellular components of the tumor ecosystem. The atlases must encompass multimodal technologies including spatial mapping technologies that preserve maximum information of the 3D tumors and their microenvironments in conjunction with an understanding of the dynamic nature of the tumors. Atlases supported by the HTA RFA must focus on one of the following transitions in cancer:

- *The transition from non-malignant to malignant disease,* including, but not limited to, atlases characterizing precancer or carcinoma in situ lesions and matched recurrent non-malignant lesions and advanced cancers from the same patients, and precancers and other atypical lesions found in surgically resected specimens or rapid autopsy specimens in association with invasive cancers.
- *The transition from locally invasive to more advanced stages and metastatic cancer*, including, but not limited to, atlases characterizing multiple metastatic sites, atlases describing the transition into or out of tumor dormancy, atlases capturing colonization of early disseminated tumor cells at distant sites.
- *The locoregional recurrence of disease within the same patient* following treatment of the primary cancer.
- *The dynamic response to therapy*, including, but not limited to, atlases describing a positive response to traditional, targeted and/or immunotherapies, atlases that describe no response, incomplete response, or negative response to traditional, targeted and/or immunotherapies.
- *The development of therapeutic resistance*, including atlases describing the transition from responsive to traditional, targeted, and/or immunotherapy to resistant to that therapy.

11. Is there a requirement to be complementary with the currently funded HTAN centers?

There is no requirement to be complementary with the existing HTA or PCA centers. It will be an open competition and we require that the applicants focus on enhancing, expanding, and adding value to the current set of HTAN atlases. Please peruse the HTAN portal (https://humantumoratlas.org/) to address this requirement. We expect the applicants to leverage the existing HTAN resources (along with their own resources) and have outlined the requirements in the RFA.

12. What is a use case in the context of this RFA?

Applicants are required to propose one or more atlas use cases within the Data Processing, Analysis, Modeling, and Visualization section of the Research Strategy. A use case is a driving question for atlas construction. The purpose of HTAN is not just to provide a catalog of cell types and locations but atlases are expected to generate/enhance biological insights poised for translation at the end of the funding period. Applicants should outline how the proposed atlas can enhance an understanding of the tumor transition(s) of focus. In addition, new hypotheses will be generated along the way and may be tested utilizing the HTAN set-aside funds required for Years 2-5.

13. Which RFA is more appropriate (HTA RFA-CA-23-039 or PCA RFA-CA-23-040) for construction of precancer atlases? How to decide?

In the case of atlases describing the precancer to cancer transition, the main differences between the two HTAN U01 NOFOs revolve around the data types for atlas construction and the purpose of the atlas.

Applicants thinking of applying to the PCA RFA (RFA-CA-23-040) should focus on generating an atlas that can guide prevention and interception strategies using streamlined technologies to analyze an adequate number of samples to satisfy the proposed atlas use case. Spatial assays can be a minor component, dissociative single-cell analysis is not required, and longitudinal sampling is desired.

Applicants thinking of applying to the HTA RFA (RFA-CA-23-039) for building a precancer atlas should focus on spatial mapping and understanding cellular interactions in a spatial context as tumors progress/evolve, use dissociative single-cell 'omics' in a supporting role, and make sure the atlas is founded upon a strong use case that can generate new biological insights.

14. Can an applicant propose a circulating tumor cell atlas that will encompass the primary and metastatic tumors as well?

The primary focus of HTAN is to build tissue-specific atlases in case of solid tumors and not on circulating tumor cells. The RFA (RFA-CA-23-039) states that "The objective of each HTA Research Center will be to construct no more than one tumor atlas that describes the molecular, cellular, and tissue-level spatial, functional, and/or temporal relationships of cellular and non-cellular components of the tumor ecosystem." If an applicant proposes to supplement the primary goal and tissue-level information with additional information gained through circulating factors/cells, that would be acceptable, but this cannot be the main goal when studying solid tumors.

15. How important is it to address heath disparity and collect samples from patients representative of the U.S. population? What if an applicant is strong in many resources and has weakness in this aspect?

All HTAN atlases should contain a diversity of patient populations. As mentioned in the RFA, priority will be given to HTAN atlases that propose a diversity of patient populations consistent with the requirements of the NIH Inclusion Policies for Research Involving Human Subjects (as described in https://grants.nih.gov/policy/inclusion.htm). NCI is particularly interested in applications where samples are collected from patients whose self-identified race and ethnicity and/or genetic ancestries are currently underrepresented in cancer research, underserved populations, or populations who experience

disparate cancer outcomes. The adequate breadth of samples included within each Center will enable future translational research based on the HTAN atlases to be relevant to and reflect all U.S. patient populations.

As stated within RFA-CA-23-039:

Following initial peer review, recommended applications will receive a second level of review by the National Cancer Advisory Board. The following will be considered in making funding decisions:

- Scientific and technical merit of the proposed project as determined by scientific peer review.
- Availability of funds.
- Relevance of the proposed project to program priorities.
- The potential for the proposed atlas to add significant value to the current sent of HTAN atlases.
- The potential for the proposed atlas to benefit the full diversity of patients within the United States.

Questions on Application Structure (Budget, Team Organization, and Page Limit)

16. What are set-aside funds, what is the purpose, and how to budget for these funds?

This is described in the *Set-Aside Funds for HTAN Pilot Projects and Trans-Network Projects (TNPs)* section of the RFA under R&R or Modular Budget. Applicants are required to set aside 15% (Direct Cost) of their annual budget in Years 2–5 to facilitate the testing of hypotheses derived from atlas construction and to develop TNPs. This is a mechanism to promote trans-network collaboration and/or provide an opportunity to develop pilot projects and enhance the research outlined in the application. These pilot and collaborative studies will be developed in the last quarter of Year 1 and will be subject to evaluation by the Steering Committee and NCI authorization. Use of the restricted funds will be contingent upon the recommendation of the Steering Committee. Examples of such projects include testing of HTAN-derived hypotheses in appropriate preclinical systems (this could include PDX, patient-derived organoids, or other mammalian systems) or validating an emerging theme (e.g., specific cell-cell interactions) from the HTAN Research Centers spanning across multiple cancer transitions and/or cancer types. The TNP topics are subject to discussion after the launch of the second phase of HTAN.

The set-aside amount should be presented in the "Other Direct Costs" category under the heading “Consortium Collaborative Funds". The use of the set-aside funds will be restricted until pilot projects and/or TNPs are developed. Do not include a description of pilot projects or TNPs within the application.

17. Are Foreign Institutions eligible for funding?

No and Yes. Foreign Institutions or non-domestic components of U.S. Organizations may not apply as the primary awardee. However, foreign components to U.S.-led applications are allowed, provided that they are justified (i.e., justified in terms of expertise).

18. Can research teams span institutions?

Yes. Team members may span multiple institutions, and it is possible that the full range of expertise needed for a proposal may not exist at one institution. Teams are expected to assemble the expertise across labs, disciplines, institutions needed to achieve the goals of the project and the HTAN.

19. Can Early-Stage Investigators (ESIs) be a PD/PI or MPI or Co-I and will that be viewed differently/favorably compared with applications from well-established/senior investigators?

It is to be noted that the HTAN applications will be reviewed by a special emphasis panel and will not be percentiled. Hence, unlike R01 applications, there is no specific advantage for the ESIs from a scoring standpoint. However, ESIs are welcome to submit an application, should these RFAs be of their interest. For an application to be viewed as an ESI application, all the listed PD/PIs should have this status at the time of submission.

As stated within the NOFO, applications from well-established/senior investigators should include funds to enhance professional development (e.g., participation in projects, attendance of meetings) of early-career/junior investigators, which includes postdoctoral fellows. Activities can include, but are not limited to, participation in cross-consortium junior investigator meetings (i.e., the NIH Junior Atlas Builders meeting).

Funding decisions will be made based on the merits of the applications identified by the peer reviewers and programmatic needs.

20. Is it acceptable to have the same contact PD/PI in two applications in response to the HTA RFA and/or PCA RFA, and can an applicant submit the same application to both RFAs?

No. As stated in the HTA RFA "An investigator designated as a Contact PD/PI of an HTA Research Center application must not be the designated Contact PD/PI of another application under this NOFO or under the companion NOFOs (RFA-CA-23-040 and RFA-CA-23-041). The Contact PD/PI can be an MPI or a Co-I on another application, whether for this NOFO or the companion NOFOs. An MPI on an HTA Research Center application may be an MPI or a Co-I on another application, whether in response to this NOFO or the companion NOFOs." The same principle applies to the companion PCA RFA.

An applicant must note that submission of duplicate or overlapping applications to both RFAs (RFA-CA-23-039 and RFA-CA-23-040) is not acceptable.

21. Is the Page Limit for the Research Strategy section 30 pages or 18 pages?

The intent is to allow more than the 12 pages allowed for standard NIH applications and therefore the RFA permits the maximum allowable limit of 30 pages. However, applicants are strongly advised to be mindful of reviewer burden and adhere to the suggested page lengths for the Research Strategy sub-sections, which add up to 18 pages. We believe that 18 pages are sufficient to capture the most important and necessary information pertinent to atlas construction.

The Specific Aims page of the PHS398 Research Plan section is not counted in this page limit.

Questions on the Outlined Functions for Atlas Construction

22. Are cancer types being prioritized in the HTA RFA?

No, cancer types are not being prescribed or prioritized. However, proposed atlases that add significant value to the current set of HTAN atlases (www.humantumoratlas.org) will be considered high priority. Significant value may be added through multiple approaches, including, but not limited to:

- Proposing tumor or tissue types not included in the first phase of HTAN;
- Proposing to include a unique sample set that diversifies or extends the impact of an atlas constructed in the first phase of HTAN; and/or
- Adding longitudinal samples that increase the insight and impact of atlases constructed during the first phase of HTAN;
- Addressing a cancer type of major public health concern and of high clinical priority, such as cancers with a clear disparity in outcome across populations.

23. What does "one 3D atlas" mean and can an applicant use one or few samples?

Each applicant should propose one atlas. The atlas could describe one cancer type, or it could describe one transition (as an example, metastasis to the brain from many cancer types or development of resistance to one treatment modality). An applicant should craft the application to maximize the information learned based upon the use case(s) proposed within the Data Processing, Analysis, Modeling, and Visualization section of the Research Strategy. Atlas size and scope will necessarily be limited by budget restrictions and applicants are encouraged to leverage additional resources when available to achieve the statistical power necessary to address the use cases proposed.

24. Should an applicant use FFPE or fresh-frozen tissue? Is there a preference for prospective or retrospective samples and is there a requirement to build a biorepository?

It is up to the applicant to propose a strong atlas construction plan based on what resources they have and technologies they will employ. As mentioned in the RFA, it is preferred that prospective and longitudinal samples from the same patients be included, when possible.

There is no requirement to contribute a portion of all samples to build a biorepository, but HTAN awardees are welcome to contribute to an ongoing trans-network project in this area.

25. Is there a requirement to meet a specific sample number?

There are no specific sample number requirements included within RFA-CA23-039. Each atlas construction effort is different and depends on the use case and the tumor transition(s) of choice being proposed in the application. An applicant needs to make sure that the study design and proposed samples are adequate for their atlas construction effort. Applicants should be sure to read and address RFA-specific review criteria included in Section V: Application Review Information.

26. Will the HTAN support projects that do not use human clinical biospecimens to construct the final proposed tumor atlas?

No. It is expected that data contained within the final tumor atlas will be derived from human clinical biospecimens, prospectively and longitudinally collected, when possible.

However, it is recognized that biospecimens from non-human models or *in vivo* and *ex vivo* platforms derived from human tumors, such as tumor organoids, patient-derived xenograft models, and other systems that maintain native tumor architecture may be useful for hypothesis testing and validating biological insights. It is to be noted that restricted funds (15% Direct Cost) for post-award activities could

be used for hypothesis testing using non-human models or any other platform and such projects should not be described within the application. (As an extension, also see the answer to Q16 above.)

27. What methods for tissue characterization are within the scope and/or preferred in the HTA RFA? Is there any particular technology and platform that HTA prefers?

Applicants must propose to collect at least two existing HTAN data types for which data and metadata standards and levels exist (at least one must be a spatial assay) to accelerate data sharing and integrative analysis (https://humantumoratlas.org/standards; https://humantumoratlas.org/explore; https://humantumoratlas.org/tools). Once this requirement is met, applicants may add any other data generation platform of their choice that will allow them to address the atlas use case proposed within the application. Study design details are up to the applicant.

28. Is it acceptable to include diagnostic imaging for atlas construction?

Yes, as long as RFA requirements are met.

29. Will HTAN-DCC help with the study design, statistics, and/or atlas construction efforts?

No. An applicant is expected to be self-sufficient and have all necessary expertise in their Research Center team to design a strong study and atlas construction plan in the application. Peer reviewers should be able to judge the proposed atlas in its full capacity. The main role of HTAN-DCC, as described earlier, is to collect, store, curate, and disseminate all data, metadata, experimental protocols and standard operating procedures, analysis and visualization tools generated by the HTAN, and conduct other Network coordination functions.

30. Does HTAN provide a core service?

No, HTAN does not offer a core service.

31. Can an applicant's existing data standards for a certain data type be remapped to the HTAN data format and is that acceptable?

Existing datasets should be remapped to the HTAN data standard if they are utilized in atlas construction. Sharing of existing datasets proposed for atlas construction, mapped to HTAN data and metadata standards, will be expected.