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PRINCIPAL INVESTIGATOR: Galit Alter PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Ragon Institute of MGH, MIT, and Harvard
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Cambridge, MA 02139

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Current HIV-specific broadly neutralizing antibodies (bNAbs), selected for their ability to recognize the virus itself, fail to sufficiently kill infected cells and have only had a very modest impact on the HIV reservoir size in humans. Spontaneous control of viral rebound does infrequently occur during natural infection (post-treatment controllers) and seems to require specific functional antibody profiles with unique antigen specificities. These antibodies shall be identified and extracted from individuals who are undergoing antiretroviral treatment interruption and be functionally optimized to enhance the rapid and highly effective deletion of virally infected cells with the goal to develop "anti-reservoir" monoclonals that may be used as stand-alone therapeutics. In three aims, this project will define the correlates of humoral immunity that track with viral remission following treatment interruption followed by development of a library of and functionally enhanced novel monoclonal antibodies poised to recognize and kill reactivated latently HIV infected cells as novel therapeutics for HIV cure strategies. From existing sample banks (The Thai Red Cross AIDS Research Centre and United States Military HIV Research Program, Walter Reed Army Institute of Research) we selected individuals who controlled or did not control viral rebound after antiretroviral treatment interruption. To define the antigen-specific titer characteristics, an array of different HIV antigens was used to measure the antigen-specific antibody isotype and IgG subclass titer in all available patients and the most relevant and targeted antigens for the functional assays were identified. While patients that initiated treatment during the chronic phase of infection developed a robust humoral immune response against the virus, early antiretroviral treatment during the acute phase of infection abolished or at least dampened such a response. Interestingly, after treatment interruption, titers stayed relatively stable potentially explained by the lacking recall of memory cells. In some individuals, however, an increase in anti-p24 and anti-gp140 IgG was detectable. Whether, this may separate the cohort into subsets of patients with differing clinical outcomes will be determined next. To further delineate the functions which track with delayed rebound/viral control, we measured the Antibody Dependent Complement Deposition (ADCD) capacity and again we observed substantial differences in complement depositing activity across the patients and across timepoints. To determine if there is a functional signature that will track with antibody responses in individuals with controlled virus after ATI, we will next examine, using system's serology, the entire spectrum of antibody functionalities, including Antibody Dependent Cellular Cytotoxicity (ADCC), Antibody Dependent Cellular Phagocytosis (ADCP), Antibody Dependent Neutrophil Phagocytosis (ADNP) etc. In addition, antibodies will be isolated from post-treatment controllers and equipped with the most promising functional properties as identified in our system's serology approach. Together, this strategy will advance the development of novel monoclonal therapeutics during the upcoming funding period.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	26	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Current HIV-specific broadly neutralizing (bNAbs), selected for their ability to recognize the virus itself, fail to kill all infected cells and have only had a very modest impact on the HIV reservoir size in humans. Spontaneous control of viral rebound seems to require specific functional antibody profiles with unique antigen specificities. These antibodies shall be identified and extracted from individuals who are undergoing antiretroviral treatment interruption and be functionally optimized to enhance the rapid and highly effective deletion of virally infected cells. These "anti-reservoir" monoclonals may be used as a stand-alone therapeutic or used to guide the development of a therapeutic vaccine able to drive a functional cure against this deadly pathogen.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

HIV, latency, HIV reservoir, treatment interruption control, broadly neutralizing antibodies, Systems Serology, HIV control.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1:	Define the correlates of humoral immunity that track with viral remission following treatment interruption
Specific Aim 2:	Develop and down select a library of novel monoclonal antibodies poised to recognize and kill reactivated latently HIV infected cells
Specific Aim 3:	Develop and test functionally enhanced "reservoir-targeting" monoclonal antibodies to cure HIV

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This study partners with investigators at the Military HIV Research Program (MHRP) to generate novel monoclonal therapeutics, that will be able to specifically target the cellular HIV reservoir, thereby supporting reservoir eradication and potentially resulting in (functional) cure of individuals infected with HIV. We selected samples from existing sample banks (The Thai Red Cross AIDS Research Centre and United States Military HIV Research Program, Walter Reed Army Institute of Research) including individuals who controlled or did not control viral rebound after antiretroviral treatment interruption. The samples are derived from studies in patients from Thailand that were treated soon after (hyper)acute HIV infection (Fiebig stage I-IV) (cohort 1) or treated in the chronic phase of the infection (cohort 2).

During this funding period we used the Systems Serology approach to define the specific correlates of humoral immunity that track with viral remission following treatment interruption **('Specific Aim 1')**. To profile the HIV-specific antibody profile in this cohort, we measured plasma Ig titers against different HIV-1 proteins across different HIV-1 strains and clades. As expected, highest titers were observed for HIV-1 gp120 and gp140 from the clade AE (CRF01), which is the dominant circulating HIV-1 clade in Thailand (see previous annual report). Titers for HIV envelope (Env) antigens from different strains within the same clade were highly correlated to each other, indicating an overall good recognition of the used antigens by plasma antibodies of the study population. To obtain an unbiased look at the antibody profiles in our study population, we applied a system serology approach to quantify binding and Fc functional antibody characteristics. Subclass and isotype titers and the antigen-specific binding potential to different Fc gamma receptors (FcγR) were measured by Luminex multiplexing. We also systematically explored the potential of the patient's antibodies to induce antibody-dependent complement deposition (ADCD), neutrophil phagocytosis (ADNP), monocyte phagocytosis (ADCP) and NK cell activation against the three selected antigens. Furthermore, we measured the NK cell mediated antibody-dependent cytotoxicity (ADCC) to HIV-1 infected lymphocytes as well as the Fc glycosylation profile of gp120 (clade AE)-specific IgG antibodies. Overall, as expected, the antibody signatures were quite heterogenous between individuals, but consistent across antigens (Fig 1A). In order to determine which factors could be involved in explaining antibody profile differences, we applied a non-supervised principal component analysis (PCA) to the entire data set and found distinct separation along PC1 for individual's with viral rebound early (<4 weeks) and delayed (≥ 4 weeks) following ATI (Fig 1B). While we observed significantly elevated levels of clade AE gp120 and gp140 specific titers for all four IgG subclasses and IgA2 in plasma of the early rebounders, delayed rebounders exhibited significantly increased binding to FcγRI and FcγRIIIa across all tested antigens when normalized to total Ig levels (Fig 1D). Interestingly, when normalized to their individual IgG titer, delayed rebounder exhibited an overall augmented humoral immune response for ADCP, ADNP, ADCC and NK cell function/cytokine induction (Fig 1E). Moreover, individuals with early viral relapse had significantly less galactosylated (more G0 structures) and sialylated IgG Fc glycans suggesting a more inflammatory antibody profile (Fig 1F). Given the substantial differences in HIV-specific antibody features between individuals with early or delayed viral rebound, we determined if the observed variation was the result of a superior host immune response versus an antigen specific process. Tetanus toxoid-specific antibody profiles, however, did not differ in functional profiles including ADCD, ADCP, ADNP and ADNKA between early and delayed HIV rebounder, confirming that the difference we had seen, were related to HIV. Of note: we were unable to detect considerable HIV specific Ab titers in individuals that initiated ART during the acute phase of infection (cohort 1) compared to our cohort of individuals with a later ART initiation (Fig. 2). Hence, early ART initiation might have suppressed an initial humoral immune response.

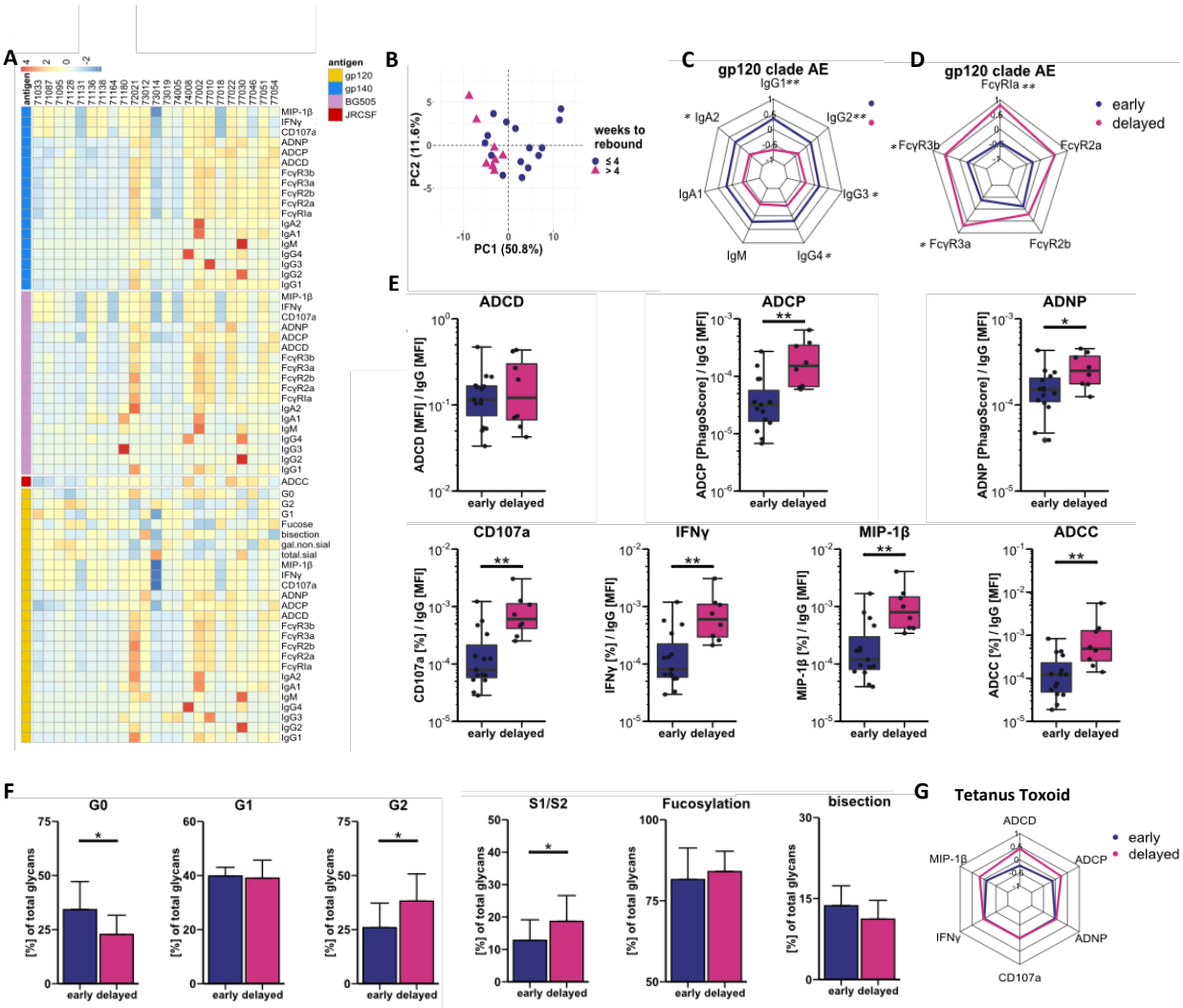

Figure 1: Comprehensive systems serology data of the tested cohort. *(A) Heatmap of Z-scored data of the antibodiome dataset, (B) Principal component analysis (PCA) of the generated antibody profile data set of HIV-specific serum antibodies before ATI. Each dot represents one individual (n=23) and was color-coded by weeks to viral rebound. (C) Radar plot of the average (after Z-scoring) antigen-specific antibody titers to gp120 AE. (D) Radar plot representation of the titer-corrected and Z-Scored antibody FcγR binding. (E) Antibody functions for gp120 clade AE specific ADCD, ADCP, ADNP and ADNKA as well as ADCC of JRCSF HIV-1 infected CEM cells before ATI were corrected for the individuals gp120-specific total IgG titer. (F) Fc glycosylation of gp120 (clade AE) specific IgG before ATI. (G) Radar plot of the average (after Z-scoring) antigen-specific antibody titers to tetanus toxoid. (*: p ≤0.05, **: p ≤0.01, ***: p ≤0.001, ****p: ≤0.0001)*

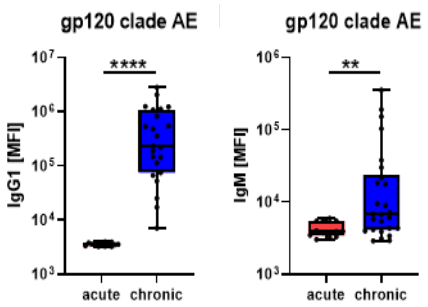

Figure 2: *Lack of HIV-specific immunoglobulins in patients who started anti-retroviral therapy early in the acute phase of infection. Gp120 (clade AE and clade B) specific IgG1 (A) and IgM (B) responses were analyzed by Luminex in HIV infected individuals who initiated ART in the acute (n=13 individuals who received placebo in the RV397 and RV411 ATI studies) or in the chronic (n=23) phase of infection (STACCATO study).*

The result from aim 1 therefore clearly show distinct antibody features differences that track with viral rebound dynamics. Given this association between humoral immune profiles and time to rebound following ATI, we applied a computational machine learning approach to identify minimal antibody features which discriminate early and delayed rebounders and, hence, might play a role in viral control but could also be developed further as marker of reservoir activity. A random forest recursive feature elimination approach was used to select a minimal number of features necessary to differentiate between early and delayed responders. The robustness and significance of the model was then assessed by comparing the actual model against models based on fold-specific size-matched random features and randomly permuted labels within a five-fold cross-validation framework. For 20 repetitions of this procedure, the model achieved an average cross-validation accuracy of 89%. Strikingly, the increased NK cell activation and engagement of FcγR2a by gp140 (clade AE) specific IgG in delayed rebounding patients was particularly important for the model, as these functions were among the top 4 selected features to discriminate early and delayed rebounder in the random forest model. In a co-correlation network analysis showing significant correlation of the selected features, NK cell activation was highly correlated for all antigens and interestingly also to cellular phagocytosis (ADCP) ($|r|>0.7$ and $p<0.05$) (Figure 3).

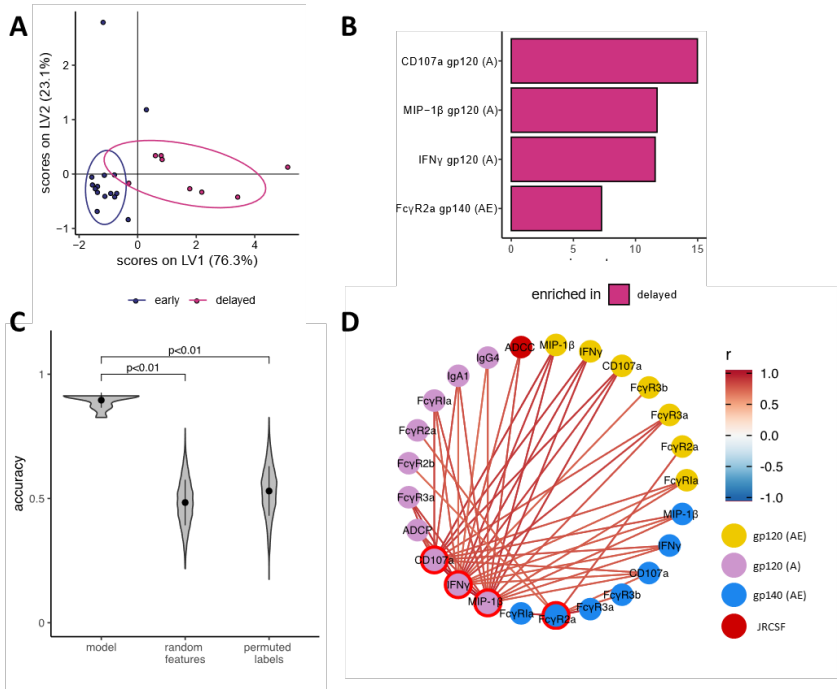

Figure 3: *(A) O-PLS-DA latent variable (LV) score plot for the features selected using a random forest recursive feature elimination approach to classify early and delayed rebounds. Ellipses show the distribution of the groups as 95% confidence levels assuming a multivariate Student's t distribution. (B) Selected features are ranked according to their importance calculated as mean decrease in accuracy and colored according to their enrichment in early or delayed rebounders. (C) Violin plot for the accuracies of the random forest model from 10 repetitions of five-fold cross-validation for the actual model and models based on size-matched random features and randomly permuted labels. For the random feature and permuted labels models 100 random permutations are performed within each of the 10 repetitions. The p-values are determined based on the probability that the accuracy of the permuted and random feature model is higher than for the actual model. (D) Co-correlates network showing features that are significantly correlated to the selected features (red border) and have a Spearman rank correlation coefficient $|r|>0.7$. Edges are colored according to their correlation coefficient, and significances were corrected for multiple hypothesis testing with the Benjamini-Hochberg procedure.*

During the second half of this funding period we started experimental work for the development and down selection of a library of novel monoclonal antibodies poised to recognize and kill reactivated latently HIV infected cells **('Specific Aim 2')**. This work was significantly delayed as all non-COVID-19 related research was put on hold at our institution from spring through summer and only recently has resumed. Conventional library generation involves baiting, flow cytometric sorting, and BCR sequencing of B cells with a specificity against soluble HIV antigens (e.g. monomeric or trimeric spike antigens). However, the monoclonal antibody library generated under this aim should be targeting HIV antigens that are displayed on the surface of infected and reactivated cells, instead of primarily binding the Env trimer on virions, and we therefore plan to use an alternative approach to identify such antibodies/BCRs. Our approach is based on a technology first described by Huang/Connors et.al. (doi:10.1038/nprot.2013.117) (Fig. 4A). IgG+ B cells from our PBMC samples are seeded into wells of 384 well plates at low cell density and cultured and stimulated for 14 days. During this period B cells proliferate and secrete their B cell receptor into the supernatant. The initial low cellular density ensures low clonal heterogeneity per well which allows us to obtain individual BCR sequences (Fig. 4B). As a next step and in contrast to the Huang/Connors et al approach which assess neutralizing activity, supernatants are harvested and screened for antibody binding to HIV-infected cells. This will be quantified by measuring antibody labeling of HIV envelope transcripts on the surface of the latency model cell lines ACH-2 [LAI] in a high throughput flow cytometric based assay. ACH-2 cells will be activated resulting in robust upregulation of p24+ staining and HIV-envelope expression (Fig. 4B). BCR sequences for library generation are then obtained from wells with positive screening result. In order to identify optimal sorting and culture conditions in a first sorting experiment, we were able to successfully culture B cells and detect IgG in the supernatant (Fig. 4C). As expected, we observed a direct association between the number of cells per well and the frequency of (IgG) positive wells. However, when the culture was started with only 4 cells/well, IgG was still detectable in more than 50% of the wells, while the number of possible B cell clones per well is low enough to obtain high quality BCR sequences.

Overall, we expect to generate a promising library of antibodies that robustly label reactivated cells using this novel selection process. Such antibodies will then be engineered for enhanced functionality in Aim 3 with the goal to generate highly efficacious monoclonal therapeutics for HIV cure strategies.

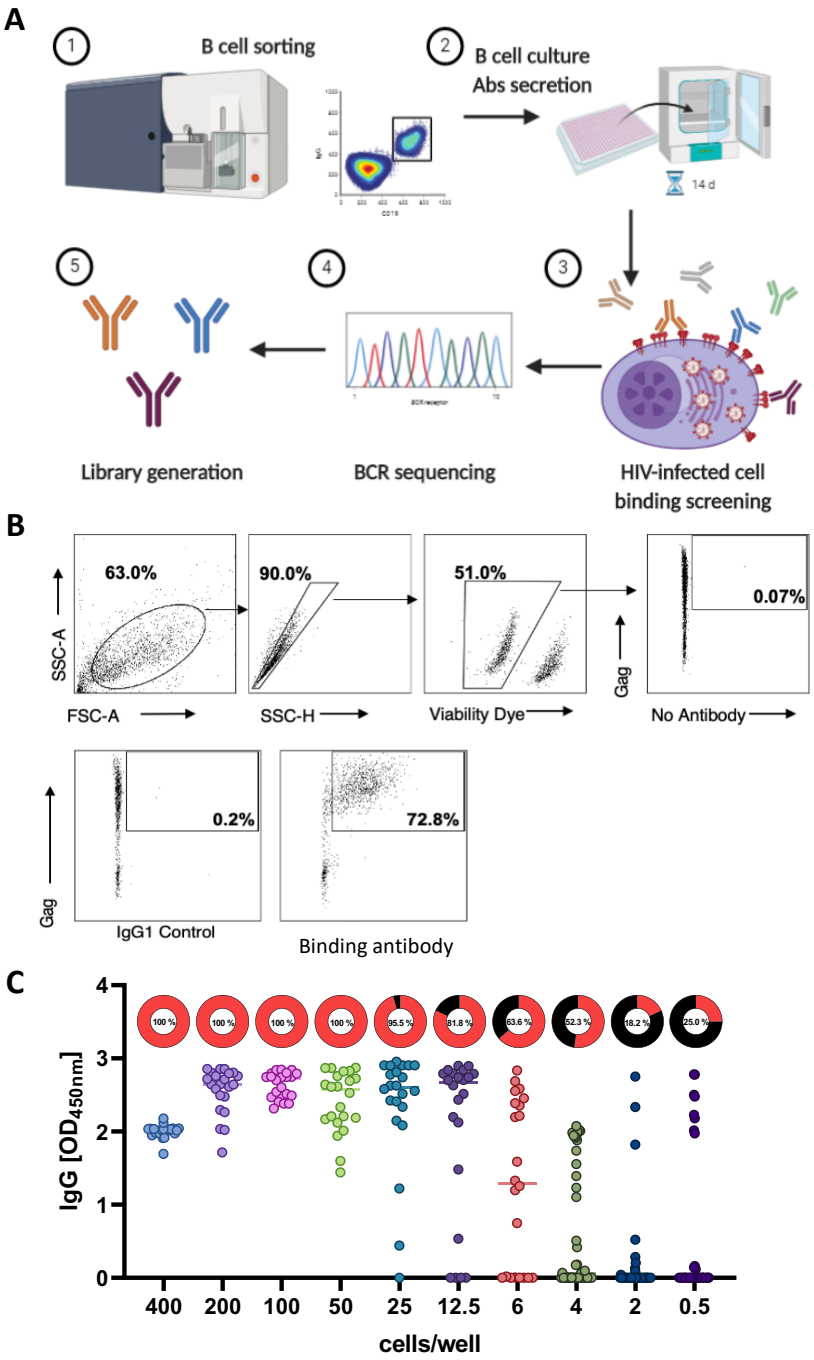

Figure 4: (A) Schema of the experimental procedure to generate a library of monoclonal antibodies with high affinity to cell surface expressed HIV antigens. (B) Flow based determination of antibody binding to activated (Gag p24+) ACH-2 cells. (C) IgG+ B cells were sorted and distributed at different cell densities (between 0.5 – 400 IgG+ B cells) into 384 wells plate. B cells were then cultured for a total of 14 days and supernatant screened for IgG levels by ELISA. Donut plots indicate the frequency of IgG positive wells per condition.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Manuscript with data from Aim 1 is under review at *mBio*. Data was also accepted for oral presentation at the HIV keystone symposium in Spring 2020, which was eventually cancelled due to the COVID-19 pandemic.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue with the experimental work in order to accomplish the goals described in aim 2 and 3. In particular we will focus on the generation of a library of monoclonal antibodies from the individuals in the available cohorts that demonstrated viral control post ART interruption and we will select for novel monoclonal antibodies with a superior ability to recognize and kill reactivated latently HIV infected cells in-vitro. In the case that we are unable to identify sufficient numbers of monoclonals from the Thai cohorts, we will create monoclonal antibody libraries from our cohorts of HIV elite controllers at the Ragon Institute for which we have detailed characterizations of serum antibody functionality – known to kill infected cells robustly- applying the same system serology approach as used here in aim 1. This will ascertain that we will have sufficient monoclonals to choose from for downstream optimization, ie Fc engineering, in vivo experiments and therapeutics development.

4. What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Plasma viremia re-occurs in most HIV-infected individuals once antiretroviral therapy is interrupted. The kinetics of viral rebound, specifically the time until plasma virus becomes detectable, differ quite substantially between individuals, and associations with virological and immunological factors have been suggested. Standard clinical measures, like CD4 T-cell counts and plasma HIV-RNA levels, however, are poor predictive markers. The antibody features identified in Aim 1, can be used as sensitive indicators of HIV disease activity and could be included in future ATI studies.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Government and hospital mandated lockdown between March and July 2020 due to the SARS-CoV-2 pandemic did not permit on-site/lab research activities.
We have been unable to perform the animal work- but are still COMMITTED to do that. The proposal specifically called for the use of humanized mice to look for reservoir killing activity and 2 delays prevented us from running these experiments yet:

1. We experienced an unfortunate delay in receiving samples from Thailand to perform the antibody profiling experiments required to guide sorting efforts. Additionally, due to the pandemic, we were unable to complete our sorts and antibody selection since March 2020- and thus have been unable to identify, downselect, engineer, and then perform animal experiments. However, this work is underway and we hope to have our final panel of antibodies in the coming months that can move forward into humanized-mice before the end of the year.
2. Secondly, in June 2019 the Trump administration banned the use of fetal tissue for research within the NIH that radiated to other institutions nationally. This led to a near complete stop in the production of humanized mice in our institute that has just restarted with Biden's reversal of the order. So we hope to perform these experiments in the fall – and will apply for an IACUC at the end of august.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Government and hospital mandated lockdown between March and July 2020 due to the SARS-CoV-2 pandemic did not permit on-site/lab research activities.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

All the experiments with human samples have been performed under IRB approved protocols.

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Title: **Viral rebound kinetics correlate with distinct HIV antibody features**
  Authors: Yannic C Bartsch, Carolin Loos, Evan Rossignol, Jesse M Fajnzylber, Anchalee Avihingsanon, Sasiwimol Ubolyam, Thidarat Jupimai, Bernard Hirschel, Jintanat Ananworanich, Douglas A Lauffenburger, Jonathan Z Li, Galit Alter, Boris Julg;
  Journal: mBio
  Status: under review
  acknowledgement of federal support: yes

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Accepted oral presentation titled: "Short-term posttreatment control of HIV correlates with distinct antibody features" at Keystone symposium on HIV pathogenesis and cure (March 2020). Event was eventually cancelled due to COVID-19.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Galit Alter, PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-7680-9215
Nearest person month worked: 0.6 CM
Contribution to Project: Dr Alter has provided oversight of the project and supervised Dr Bartsch in design and execution of the sample analysis
Funding Support: Complete only if the funding support is provided from other than this award.)

Name: Boris Juelg, MD PhD
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID): 0000-0002-4687-9626
Nearest person month worked: 1.5 CM
Contribution to Project: Dr Juelg has provided oversight of the project and supervised Dr Bartsch in design and execution of the sample analysis
Funding Support: Complete only if the funding support is provided from other than this award.)

Name: Yannic Bartsch, PhD
Project Role: Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-3844-3056
Nearest person month worked: 7.7 CM
Contribution to Project: Dr Bartsch is performing the experiments.
Funding Support: Complete only if the funding support is provided from other than this award.)

Name: Evan Rossignol, PhD
Project Role: Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-5347-4301
Nearest person month worked: 4 CM
Contribution to Project: Dr Rossignol is developing the B-cell screening technology
Funding Support: Complete only if the funding support is provided from other than this award.)

Name: Hacheming Compere
Project Role: Research technician
Researcher Identifier (e.g. ORCID ID): 0000-0002-0774-8623
Nearest person month worked: 8.5 CM
Contribution to Project: Mr Compere is supporting the experimental work, ie conducting experiments, sample
management etc related to this project.
Funding Support: NA

Name: Kang, Jaewon
Project Role: Research technician
Researcher Identifier (e.g. ORCID ID): 0000-0003-0846-4611
Nearest person month worked: 1.0 CM
Contribution to Project: Mr Kang is supporting the experimental work, ie conducting experiments, sample

Name: Yuan, Dansu
Project Role: Research technician
Researcher Identifier (e.g. ORCID ID): 0000-0003-4815-7237
Nearest person month worked: 1.0 CM
Contribution to Project: Mrs Yuan is supporting the experimental work, ie conducting experiments, sample
management etc related to this project.
Funding Support: NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report." If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

South East Asia Research Collaboration in HIV (SEARCH) Thai Red Cross AIDS Research Centre: This subcontract has not performed any work since the inception of this award. Dr. Eugène Kroon has not devoted any effort and no billable work has been performed. We therefore are terminating the subcontract agreement with this organization.
Other Support forms for Drs. Alter and Juelg are provided below.

OTHER SUPPORT

ALTER, GALIT

ACTIVE

OPP1146996 (Montefiori) 07/01/16 – 06/30/21 0.12 calendar (1%)
Bill and Melinda Gates Foundation $434,783
Vaccine Enterprise Comprehensive Antibody Vaccine Immune Monitoring Consortium
This project aims to establish and develop high-throughput, standardized assays to profile vaccine-induced antibody responses
Role: Co-Investigator

OPP 1151840 (Alter) 11/30/16-06/30/21 0.12 calendar (1%)
Bill and Melinda Gates Foundation $2,984,508
Defining Humoral Correlates of Mycobacterial Immunity in TB Resistors
The goal of this project is to identify unique humoral immune responses in individuals who resist TB infection that may contribute to protection from infection or progression of disease in an effort to guide future vaccine design.
Role: PI

OPP 1156795 (Alter) 11/30/16-06/30/21 0.12 calendar (1%)
Bill and Melinda Gates Foundation $1,277,896
Protective role of monoclonal antibodies in the control of MTB infection
The goal of this project is to identify the potential causal role of antibodies in MTB control in vivo in an effort to guide future vaccine design.
Role: PI

OPP1097381 (Alter) 11/14/13 – 10/31/20 0.12 calendar (1%)
Bill and Melinda Gates Foundation $984,969
A Genetic Approach to Optimizing the Antigenicity of HIV-1 Envelope Immunogens
The goal of this program is to develop a glyco-optimized HIV envelope producing cell line that is antigenically enhanced and can induce long lived B and T cell immunity.
Role: PI

(NEW)
INV-001650 (Alter) 11/21/19 - 09/30/21 0.12 calendar (1%)
Bill and Melinda Gates Foundation $847,660 (cumulative totals costs)
GH-VAP: Systems serology platform
The goal of this project is to establish The Alter Laboratory as part of The Gates Foundation Global Health Vaccine Accelerator Platforms (GH-VAP). Previously established/qualified Systems Serology assays will be made available to The Gates Foundation collaborators/community to define the role of the innate immune system in recruiting antibodies to provide protection from infection across multiple pathogens.
Role: PI

(Barouch) 05/01/16 – 04/30/21 0.24 calendar (2%)
NIH 1UM1AI124377-04 $1,199,399 (cumulative total costs)
$143,678 (annual direct costs)
Consortia for Innovative AIDS Research in Nonhuman Primates/Humoral Immunology Core
The goal of this program is to define the immunological mechanisms that lead to prevention and eradication in the non-human primate model of HIV.
Role: Co-Investigator

(Barouch) 07/01/16 – 06/30/21 0.6 calendar (5%)
NIH UM1-1AI126603-04 $1,804,713 (cumulative total costs)
$250,000 (annual direct costs)
Combined Immunologic Approaches to Cure HIV-1 - Focus 2
The goal of this project is to develop next-generation “killer” monoclonal antibodies to aggressively purge the HIV reservoir following reactivation.
Role: Focus 2 Leader

(Barouch) 07/01/16 – 06/30/21 0.6 calendar (5%)
NIH UM1AI126603-04 $717,922 (cumulative total costs)
$100,000 (annual direct costs)
Combined Immunologic Approaches to Cure HIV-1 – SRS Core
The goal of this project is to profile the vaccine-induced humoral immune responses induced by therapeutic vaccination in both non-human primates and humans to define the reservoir-depleting activity of these responses.
Role: Immunology SRS Leader

(Alter) 11/15/16 – 12/31/20 0.12 calendar (1%)
Gilead Sciences $2,828,403 (cumulative total costs)
$589,132 (annual direct cotss)
Development of a novel class of broadly functional antibodies (bFAbs) that can kill the viral reservoir within lymphoid sanctuaries
Role: PI

1R01DA043263-03 (Sekaly) 09/15/16 – 05/31/21 0.36 calendar (3%)
NIH $809,574 (cumulative total costs)
$125,000 (annual direct costs)
An unbiased OMICs approach to identify mechanisms of Cocaine regulation of the HIV reservoir
The goal of this project is to determine the impact of cocaine use on altering antibody effector functions that may disrupt humoral immune control of the HIV viral reservoir.
Role: Co-Investigator

U01 AI131296-03 (Deeks) 04/01/17 – 03/31/22 0.12 calendar (1%)
NIH $106, 307 (cumulative total costs)
$30,545 (annual direct costs)
Therapeutic vaccination and PD-1 blockade in treated HIV disease
The goals of this program are to define the safety and immunogenicity of novel DNA vaccine in HIV-infected adults on antiretroviral therapy and to determine if DP-1 inhibition during vaccination improves immunogenicity.
Role: Co-Investigator

1 R01AI129797-03 (Barouch) 08/01/17 – 07/31/22 1.08 calendar (9%)
NIH $1,159,220 (cumulative direct costs)
$149,416 (annual direct costs)
Optimization of Broadly Neutralizing Antibodies for HIV Eradication
The goal of this project is to develop a bi- or tetra-specific Fc-engineered single monoclonal antibody the covers global viral diversity and drive the rapid and highly effective clearance of reactivated latently infected cells to test in vivo in NHP following viral reactivation.
Role: Co-PI

1U01AI131295-03 (Sekaley) 03/01/17 – 02/28/21 0 calendar (0%)
NIH $172,876 (cumulative total costs)
$64,170 (annual direct costs) [work on hold]
Resetting immune homeostasis: a non-invasive approach towards HIV eradication
The goal of this project is to define the HIV-specific and natural-antibody biomarkers that track with effective reconstitution of T cells for HIV gene therapy cure strategies.
Role: Co-Investigator

U19AI135995-02 (Anderson) 02/01/18 – 01/31/23 0.12 calendar (1%)
NIH $655,732 (cumulative total costs)
$65,000 (annual directs costs)
Consortium for Viral systems Biology (CViSB)-Project 1
The goal of this project is to use an integrated systems-level identification and investigations of the host determinants of patient outcomes in Lassa virus and Ebola virus infection.
Role: Co-Investigator

U19AI128751-03 (Barouch) 02/01/18 – 01/31/23 0.96 calendar (8%)
NIH/ IPCAVD 4
$1,683,652 (cumulative total costs)
$202,856 (annual direct costs)
The goal of this IPCAVD program is to develop Ad26/Env vaccines for HIV-1 by a highly collaborative and multifaceted research program involving leading investigators in academia and industry. (P1: 0.24 CM, Core B: 0.24 CM)
Role: Co-Investigator

W81XWH1810579 (Alter) 09/30/18 – 09/29/21 0.48 calendar (4%)
DoD $1,754,195 (cumulative total costs)
$400,000 (annual direct costs)
Immune Correlate + Guided Design of Monoclonal Therapeutics for HIV Remission
The goal of this project is to define the specific antibody profile that tracks with cellular reservoir control
Role: PI

R01AI137164-01 (Harris/Charles) 09/14/18 – 08/31/23 0.12 calendar (1%)
NIH/NIAID $3,865,697 (cumulative total costs)
$6,311 (annual direct costs)
Antibody mediated protective immunity against cholera
This project aims to improve our understanding of host-pathogen interactions during cholera, result in better immunologic correlates of vaccine protection.
Role: Co-Investigator

U19AI42790-01 (Saphire) 05/01/19 - 04/30/24 0.96 calendar (8%)
NIH/NIAID $242,973 (cumulative total costs)
Consortium for Immunotherapeutics against Emerging Viral Threats - Core D
The goal of this project is to use systems serology and antibody engineering to improve against filovirus, arenavirus, and alphavirus.
Role: Co-PI

(NEW)
R37AI80289-11 (Alter) 01/09/20 – 12/31/24 0.72 calendar (6%)
NIH/NIAID $3,292,800 (cumulative total costs)
$400,000 (annual direct costs)
Demystifying the antiviral activity of the IgG3+ antibody response
This project explores and defines the specificity/functionality of IgG3+ B cell responses and the mechanism, by which the immune system programs such potent antiviral humoral immunity.
Role: PI

75N93019C00052 (Ross) 05/01/19 – 04/30/24 0.6 calendar (5%)
NIH/NIAID $194,889 (cumulative total costs)
$116,005 (annual direct costs)
Center for Influenza Vaccine Research in High Risk Populations
This project researches immunological correlates of protection in influenza infection in naturally infected human populations and in human and animal challenge models post vaccination.
Role: Co-Investigator

(NEW)
5U19AI128910-02 (Haddad) 06/01/20 – 05/31/21 0.12 calendar (1%)
NIH $59,060 [ADMIN SUPPLEMENT]
Integration of Systems Serology with OMICs analysis to generate models of vaccine response in endemic areas of parasitic infection.
To analyze the functionality of HepB antibody responses in helminth-infected subjects to determine the impact of Schistosoma infection on the qualitative features of the humoral immune response.
Role: Co-Investigator

(NEW)
75N93019C00071 (Fortune) 09/30/19 – 02/28/23 0.96 calendar (8%)
IMPAC-Tb $2,396,393 (cumulative total costs)
NIH/NIAD $475,000 (annual direct costs)
Immune Mechanisms of Protection against Mycobacterium Tuberculosis Center (IMPAc-TB)
The goal of this proposal is probe the potential biological role of antibodies in driving anti-microbial control and to generate antibodies and engineer monoclonals to further dissect the mechanistic role of antibodies in bactericidal activity.
Role: Co-Investigator

(NEW)
R01AI146785 (Alter) 03/11/20 – 02/28/25 0.48 calendar (4%)
NIH/NIAID $2,949,879 (cumulative total costs)
$344,690 (annual direct costs)
Defining the Fc-correlates of protection against influenza
The goal of this proposal is to define the influenza-specific Fc-humoral profiles that associate with protection against influenza and the development of neutralizing antibody breadth to guide next generation design of a universal influenza vaccine.
Role: PI

(NEW)
1R01AI152158-01 (Alter) 05/06/20 - 04/30/25 0.96 calendar (8%)
NIH $4,601,302 (cumulative total costs)
$708,419 (annual direct costs)
Multiplexed Antigen-Specific Antibody Fc Profiling on a Chip for Point-of-Care Diagnosis of TB in HIV-infected Children
The goal of this proposal is to develop an inexpensive, simple, reliable TB-specific antibody-based point-of-care diagnostic to manage TB disease in children under the age of 5.
Role: PI

(NEW)
3R37AI080289-11S1 (Supplement) (Alter) 04/10/20 – 03/31/22 0.48 calendar (4%)
NIH $773527 (cumulative total costs)
$230,216 (annual direct costs)
Demystifying the antiviral activity of the IgG3+ antibody response
This proposal seeks to define the humoral correlates and mechanisms of action against COVID-19 in mice, ferrets, and monkeys.
Role: PI

(NEW)
PATH (Alter) 06/01/20-12/31/22 0.12 calendar (1%)
Ab mediated innate immune mechanisms
This project aims to define the independent or synergistic function of C-term-specific antibodies.
Role: PI

(NEW)
Massachusetts Consortium on Pathogen Readiness (Alter & Barouch) 05/01/20 – 04/30/21
0.06 calendar (0.5%)
Systems based Fc-engineering to accelerate therapeutic monoclonal antibody design to COVID-19
In this project, we propose to apply our Systems Fc-Engineering approach to develop a library of functional CR3022 pan-CoV antibodies. $195,834
Role: Lead PI

(NEW)
Massachusetts Consortium on Pathogen Readiness (Knipe) 05/01/20 – 04/30/21 0.06 calendar (0.5%)
Herpesviral Recombinant Vectors for Vaccine Vectors and Study of Coronaviral Pathogenesis
$27,663
Role: Co-PI

(NEW)
2P30AI060354 08/01/20 – 07/31/21 0.6 calendar (5%)
NIH $3,806,528 [overall program]
Harvard University Center for AIDS Research
Facilitate and expand synergistic multidisciplinary collaborations among diverse and highly successful HU-affiliated HIV/AIDS researchers and their trainees, locally and internationally. Support innovative research initiatives capable of more effectively addressing key HIV/AIDS-related research priorities aimed at mitigating the effects of infection and bringing an end to the epidemic. Engage, support and mentor the next generation of Early Career Investigators in HIV/AIDS research.
Role: Co-Director

(NEW)
1R561AI155149-01 (Alter) 09/01/20 – 08/31/21 0.36 calendar (3%)
NIH $534,845
Mapping and dissecting the role of antibodies in Mtb control
This proposal aims to define the specificities, functional profiles, and anti-microbial mechanism(s) of antibodies that prevent progression to TB.
Role: PI

(NEW)
A08529 (Gupta) 06/24/20 - 12/31/20 0.12 calendar (1%)
International AIDS Vaccine Initiative (IAVI)
Systems Serology and Antibody Effector Function Cumulative Costs: $119,196
Dr. Alter will develop assays to evaluate induction antibody-mediated effector functions from NK cells, monocytes, and neutrophils against antigenic targets from Lassa virus.1
Role: Co-Investigator

OVERLAP:
There is no scientific or budget overlap between the funded grants.

OTHER SUPPORT

Juelg, Boris

ACTIVE

Ragon Institute Strategic Initiative (Juelg) 09/01/15 – 09/30/21 0.12 calendar
Ragon Institute of MGH, MIT & Harvard $751,309 cumulative total costs
($745,631 total direct annually)

A Phase 1 Study to Evaluate the Safety/Tolerability and Immunogenicity of a Heterologous Ad26 Mosaic and MVA Mosaic Vector Vaccine Regimen in Virologically Suppressed HIV-1 Infected Adults on cART
This study is proposed to be a new module in the Ragon Clinical Trials Initiative and would enable us to explore the Ad26/MVA mosaic vaccines in HIV-1-infected individuals.
Role: PI

Grant ID # PA-HIV-16-0061 (Alter) 11/15/16 –12/31/20 0.6 calendar
Gilead Sciences $2,828,403 cumulative total costs
($640,017 total direct annually)

Development of a novel class of broadly functional antibodies (bFAbs) that can kill the viral reservoir within lymphoid sanctuaries
The overall goal of this project is to develop an innovative monoclonal therapeutic strategy to cure HIV.
Role: Co-Investigator

W81XWH1810579 (Alter) 09/30/2018 – 09/29/21 0.6 calendar
DoD $1,754,195 cumulative total costs
($377,341total direct annually)

Immune Correlate + Guided Design of Monoclonal Therapeutics for HIV Remission
The goal of this project is to define the specific antibody profile that tracks with cellular reservoir control to rationally design a therapeutic strategy able to effectively eliminate the viral reservoir and drive viral remission at a global level.
Role: Co-Investigator

R01AI138790-01A1 (Juelg) 12/17/18 --11/30/23 4.21 calendar
NIH/NIAID $3,368,976 cumulative total costs
($406,432 total direct annually)

Optimizing HIV-specific T-cell responses by therapeutic vaccination
proposal examines the potential of a novel vaccine
strategy to enhance the body's immune response to better control the virus and destroy HIV infected cells.
Role: PI

Ragon Institute SOSIP Trial Award 12/01/18 -- 01/31/21 2.4 calendar
Ragon Institute of MGH, MIT & Harvard $722,171 total cumulative costs
($302,396 total direct annually)

A randomized, double-blinded, placebo-controlled, dose-ranging phase 1 clinical trial to evaluate the safety and immunogenicity of recombinant HIV Envelope protein BG505 SOSIP.664 with AS01B adjuvant in healthy, HIV-1 uninfected adults
Role: PI

U19AI142790 (Saphire) 05/01/19 - 04/30/21 0.23 calendar
La Jolla Institute for Allergy & Immunology $7,656 cumulative total costs
($4,557 total direct annually)
Advanced Development of Antiviral Immunotherapeutics against Key Viral Threats
The overall goal is to move promising antiviral monoclonals without delays into early product development.
Role: Co-Investigator

(NEW)
1U01AI145801 (Barouch; Boris) 04/01/20 – 03/31/23 3.6 calendar
NIH/NAID $361,735
($71,773 total direct annually)
Ad26 Based Therapeutic Vaccines for HIV
The overall goal is to evaluate therapeutic vaccination in combination with latency reversal agents and broadly neutralizing antibodies for HIV eradication strategies
Role: PI

OVERLAP:
There is no scientific or budget overlap between the funded grants.

Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

From The Henry Jackson Foundation:
Organization Name: HIV Netherlands Australia Thailand (HIV-NAT)
Location: Bangkok, Thailand
Contribution: In-kind support to provided clinical samples and obtain necessary regulatory approvals.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable

QUAD CHARTS: Not applicable

APPENDICES: