SUMMARY STATEMENT

PROGRAM CONTACT: [redacted]
Stacy Ferguson

(Privileged Communication)

Release Date: **07/10/2019**
Revised Date:

Application Number: **1 U01 AI148119-01**

Principal Investigators (Listed Alphabetically):

MEYER, AARON SAMUEL (Contact)
NIMMERJAHN, FALK

Applicant Organization: UNIVERSITY OF CALIFORNIA LOS ANGELES

Review Group: **ZAI1 SB-I (S1)**
National Institute of Allergy and Infectious Diseases Special Emphasis Panel Fc-Dependent Mechanisms of Antibody-Mediated Killing (U01 Clinical Trial Not Allowed)

Meeting Date: **06/20/2019**
Council: **OCT 2019**
Requested Start: **12/01/2019**

RFA/PA: **AI18-042**
PCC: **I2M**

Project Title: **Mapping the effector response space of antibody combinations**

SRG Action: **Impact Score:** [redacted]
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested	Estimated Total Cost
1	[redacted]	[redacted]
2		
3		
4		
5		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1U01AI148119-01 Meyer, A

RESUME AND SUMMARY OF DISCUSSION: This outstanding application entitled “Mapping the effector response space of antibody combinations” was submitted in response to RFA-AI-18-042, Fc-Dependent Mechanisms of Antibody-Mediated Killing (U01 Clinical Trial Not Allowed), by The Regents of the University of California, Los Angeles with Dr. Aaron Meyer as the contact Program Director/Principal Investigator (PD/PI). Dr. Falk Nimmerjahn is the other PD/PI (MPI) in this multiple PD/PI application. The proposed project will investigate the hypothesis that IgGs of identical antigen binding, but different isotype or glycosylation status, can show synergistic effector-elicited cell killing and that a multi-IgG Fc binding model can effectively identify these combinations. Three specific aims are proposed to test the overreaching hypothesis. Aim 1 will validate a multivalent binding model’s ability to predict FcγR binding to mixed IgG composition immune complexes. Aim 2 will map human and murine IgG isotypes to one another according to conserved effector response. Aim 3 will link IgG effects and in vivo efficacy to identify and verify synergistic IgG-elicited cell killing.

The proposed studies are poised to develop models that will predict antibody-mediated effector functions of antibody combinations. If successful, these studies will likely facilitate the engineering of IgG with optimal effector cell killing response and will inform how existing therapeutic and endogenous IgGs function. In this application, the multivalent binding model and the homology map correlating species may predict antibody efficacy in vivo and more accurately predict human effector responses. Therefore, proposed studies are significant. However, reviewers indicated that direct in vitro studies to determine the mechanisms for ADCC-dependent cell killing may not be feasible due to focus on immune complexes. Some of the reviewers noted that many of the proposed studies will be conducted in mice, while the potential for translation to human immune responses is not clear. Also, there is a lack of preliminary data identifying significant synergistic or antagonistic IgG pairs. A distinct strength of this application is the investigators. The investigative team is constituted by highly collaborative, accomplished scientists with considerable complementary expertise. The contact PD/PI, Dr. Meyer, is an outstanding young investigator with research focus on the interface of computational biology and experimental biology. He has a strong record of publications. The other PD/PI, Dr. Nimmerjhan, is a distinguished leader in the field of Fc-mediated effector functions. Drs. Meyer and Nimmerjhan jointly published an article on the development and parameterization of the basic model that is used in the proposed studies. The multiple PD/PI leadership plan is adequate. The scientific environment at The Regents of the University of California, Los Angeles and Friedrich-Alexander University Erlangen-Nuremberg, Bavaria, Germany is exceptional. Both of the institutions and laboratories are well positioned to conduct the computational and experimental studies as suggested in the application. The investigators proposed an innovative model to predict the strength of Fc-mediated effector function. The integration of modeling and experimental research in creating a data-driven model is innovative. The idea of the identification of synergy or antagonism in Fc receptor elicited killing is compelling. However, it is not clear whether the approach will allow the identification of synergistic or antagonistic IgG pairs. The reviewers noted that the multivalent binding model for mixed IgG composition immune complexes is a refinement of existing model for homogenous immune complexes. The proposed studies will likely provide a generalized approach in predicting Fc-gamma receptor mediated effector function in different species. Approaches in Aim 1 will expand the basic model to mixed IgG compositions that may represent the polyclonal antibody responses in vivo. In Aim 2, the mapping of IgG subclass functions in different species is a strength. However, there are some weaknesses in the approach. In Aim 1, TNP-conjugated BSAs utilized to produce immune complexes are heterogenous, which may introduce additional variability into the proposed complex model. A weakness in Aim 2 is the proposition of linking murine and human data based on the assumption that similar profile will be observed in mouse and human. Also, Aim 3 anticipates humanized mouse will represent human immune responses. Moreover,

insufficient description is provided to distinguish the contribution from mouse and human parts of the humanized mouse model. In proposed studies, the challenges in the measurement of binding of the multivalent immune complexes in the picomolar range is not adequately described. Overall, there is enthusiasm for the project's significance, innovation, the outstanding investigative team and environment. However, enthusiasm is somewhat reduced by weaknesses in some of the approaches, insufficient supportive preliminary data and unclear translation of data from mouse model to human immune responses. Based upon the evaluation of scientific and technical merit, this application received an Impact/Priority score of [redacted].

DESCRIPTION (provided by applicant): Antibodies are crucial, central regulators of the immune response. They are particularly versatile therapeutic agents due to their ability to both bind to a target with high affinity and direct the immune system. Indeed, antibodies comprise a broad range of approved therapies across disease indications, many of which are known to rely in large part on effector cell (immune) response. Antibodies of the IgG isotype interact with FcγRs on effector cells and elicit effector function through multiple cell types (e.g., macrophages, monocytes) and through multiple processes, including phagocytosis and killing of diseased cells. The many possible design parameters—constant region composition, FcγRs, cell populations, and antigen binding in combination—have made precisely understanding, measuring, and manipulating effector function an elusive goal. Our proposed work is centered around the hypothesis that two IgGs can elicit distinct responses when present in combination from what would be suggested by the response to either on its own. Using a computational model of antibody-FcγR interaction, we will identify predicted cases of this emergent behavior. These combinations will be tested for their binding and effector response in vitro and then in two models of antibody-targeted cell killing. Finally, we will use the computational model of effector regulation to map how human and mouse IgGs are related according to their effector response. In total, these efforts will provide critical information for designing more effective antibodies with the goal of targeted cell killing and provide a clearer view of how existing therapeutic antibodies function.

PUBLIC HEALTH RELEVANCE: Antibodies, especially those of the IgG type, are central to immunity and comprise a wide class of biologic therapies. In addition to binding an antigen target, IgG antibodies direct the response of immune cells through Fc-gamma receptor binding. This project aims to better understand how antibodies influence the behavior of one another in target cell killing when present together.

CRITIQUE: The comments in the CRITIQUE section were prepared by the reviewers assigned to this application and are provided without significant modification or editing by staff. They are included to indicate the range of comments made during the discussion, and may not reflect the final outcome. The RESUME AND SUMMARY OF DISCUSSION section summarizes the final opinion of the committee after the discussion and is the basis for the assigned Overall Impact/Priority score.

CRITIQUE 1

Significance:	3
Investigator(s):	2
Innovation:	4
Approach:	5
Environment:	1

Overall Impact: This project focuses on building models to predict antibody-mediated effector functions of antibody combinations. These models will then be utilized with in vitro and in vivo experiments to identify antibody combinations that act synergistically or antagonistically to activate/inhibit effector

functions. As part of the project a homology map to link human and murine IgG isotypes according to effector functions elicited will be produced. This homology map will be used to allow results from studies of antibodies in mouse models of disease to be more effectively translated to humans. Both of the PD(s)/PI(s) of the application have research and publications records related to mechanisms of Fc-dependent, antibody-mediated killing. The multivalent binding model and the homology map correlating species have the potential to enable predictions of antibody effectiveness in vivo and to more accurately predict human effector responses based on mouse disease model studies. The preliminary results and references cited demonstrate initial model building based on immune complexes made up of a single antibody form, and this project will extend these studies to mixtures of antibody forms. However, there is a lack of data for synergistic or antagonistic antibody combinations. Therefore, it is unclear how significant the synergistic/antagonistic effects of immune complexes of antibody combinations will be and if the approach presented will identify those. The approach to generating data for modeling, including heterogeneity of the multivalent immune complexes and methodology used in binding studies, may reduce the rigor of the resulting models. Overall the studies described will likely increase knowledge on how mixtures of antibodies activate effector cells and may identify pairs of antibodies that interact to alter effector functions.

1. Significance:

Strengths

- The proposed experiments will study how immune complexes (anti-TNP antibodies bound to TNP conjugated BSA) made up of mixtures of IgG isotypes or ±fucose glycoforms effect cell binding and activation in an effort to understand how different IgG isotypes and glycoforms combine in vivo to elicit cell killing.
- In vitro binding and activation experiments with immune complexes made up of IgG mixtures will be used to build multivalent binding models that may predict in vivo cell killing.
- In vivo mouse experiments will be conducted to test the use of the multivalent binding models in predicting IgG-mediated cell killing.
- In the in vivo mouse models of cell killing described in aim 3, immunothrombocytopenia and cytotoxic antibody-mediated B cell depletion, the effector cells may be responsible for cell killing are liver resident Kupffer cells and/or resident monocytes.

Weaknesses

- Due to the reliance on immune complexes, direct in vitro studies for the mechanism of ADCC-mediated cell killing may not be possible.
- The binding/effector models presented in the application do not consider reorganization of receptors during immune synapse formation and the effects it may have on ADCC/ADCP.
- The binding/effector models presented in the application focus exclusively on Fc-FcR interactions and do not consider the contribution of other ligands at the cell-cell interface, such as ICAM-1 and LFA-1, to ADCC/ADCP.
- One of the assumptions of the multivalent binding model is uniform affinity for IgG of a certain type. The production methods described will produce heterogenous glycosylation and potential chemical modifications. These modifications will result in a range of receptor affinities for the resulting mixture of each IgG type, with the affinity differences potentially being significant enough to impact the model (at least in the case of FcRIIIa binding).

2. Investigators:

Strengths

- The investigators have previously published research on Fc-dependent, antibody-mediated killing and have expertise relevant to the proposed specific aims.

Weaknesses

- None were noted.

3. Innovation:

Strengths

- The effects of immune complexes containing mixtures of IgG isotypes or ±fucose glycoform mixtures will be assessed to identify synergy or antagonism in Fc receptor elicited killing.
- This research will develop a multivalent binding model for mixed IgG composition immune complexes that will be combined with effector cell FcγR expression levels to attempt to predict antibody effectiveness in vivo.
- A homology map correlating human and murine IgG isotypes will be created to allow results obtained from mouse disease models to predict IgG effects in humans using the hypothesis that there is conserved regulation across species of cell type-specific effector responses.

Weaknesses

- It is not certain that significant synergistic or antagonistic IgG pairs will be identified using this approach (none are identified in the preliminary results) or if pairs might just as easily be identified by ranking monomeric IgG subclass/glycoform affinity for different receptors.
- If synergistic or antagonistic IgG pairs are identified, it is not obvious how this knowledge could be utilized to produce therapies since regulatory and drug development considerations favor single active molecules.
- The multivalent binding model for mixed IgG composition immune complexes is a refinement of their existing published model for homogenous immune complexes.

4. Approach:

Strengths

- The binding measurements conducted on immune complexes enable measurements of multivalent Fc receptor interactions that mimic interactions likely to occur at the interface of target and effector cells.

Weaknesses

- The TNP-conjugated BSAs utilized to produce immune complexes are not homogeneous with TNP valencies of 4 and 26 as described in the application but heterogeneous protein conjugates produced by random amine conjugation chemistry, introducing additional variability into the complex models.
- Kinetic lability of non-covalent binding of anti-TNP antibodies to TNP-BSA and engagement of one or two Fab arms per antibody will result in heterogeneity in the number of antibodies in immune complexes.
- There is a lack of consideration of difficulties involved in measuring tight binding (binding in the picomolar range of KDs or lower), where very low concentrations of ligands and long incubation times to reach equilibrium are required to accurately measure equilibrium dissociation constants. The methods and references describe that if immune complexes with sub-nanomolar

dissociation constants were present (as might be expected for multivalent immune complexes made up of non-fucosylated IgG1 binding to FcRIIIa), the methods would result in binding measurements being made far from equilibrium resulting in overestimation of the dissociation constants and skewing of the resulting models built from those measurements.

5. Environment:

Strengths

- The scientific environment is appropriate.

Weaknesses

- None were noted.

CRITIQUE 2

Significance:	2
Investigator(s):	1
Innovation:	3
Approach:	3
Environment:	1

Overall Impact: This application suggest a combined experimental approach that untypically starts with a data driven modeling approach, which will then be modified and expanded through experimentation. If successful it should create a novel dynamic map FcγR and IgG composed immune complexes, which will be related to sets of prescribed cellular functions and most accurately to IgG elicited cell killing. This model/construct could have a great impact both on our understanding of the impact of multivariate activation and function and if the relationship between murine and human responses is elucidated also on our ability to design cocktails of effective antibodies. However, it is unclear how the modeling schemes ability to be extend to multivariate responses, or how the investigators will relate human and murine responses if those express different patterns or a combination of similar and species individual relationships.

1. Significance:

Strengths

- The application proposes to create a data driven model of the types of multivariate interactions between FcγR and IgG composed immune complexes in mice.
- The investigators will then link these to effector responses and specifically their relationship to IgG-mediated cell killing in both mice and humans.

Weaknesses

- If the existing tools and proposed older modeling techniques may not lead to new insights, beyond what the investigators have already used these tools to learn. Also, it is not clear what alternate methods would be used.
- Minor - most of the results are in the murine model, which may not be as easily translatable to human immune responses as suggested. To be clear, the murine study on its' own would be of great significance. Nevertheless, the promise of Aim 2 to link findings between species depends on their being clear points of overlap that share both their core determinants and their borders. However, it is not clear to what extent any relationship will be presented or insight gained regarding humans.

2. Investigators:

Strengths

- Both PIs have a long standing record of collaboration as well as specifically collaboration on topics related to the study proposed.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Innovation rests on the idea of combining modeling and experimental research to create a data driven model. This model generates predictions, which can be tested and fed back into the model.
- This application suggests a modeling and experimental schema that will allow to model multivariate receptor response and relate them to cellular function and cell dynamics outcomes.

Weaknesses

- The modeling schemas used are either dated or simply minor refinements of the investigators' existing tools. Aim 1 proposes that the investigators will use their published modeling schema without adding any parameters. There is a lack of consideration of alternative tools that would allow them to continue the analysis if existing tools are not adequate. This is a problem as the multivariate nature of the IgG response potentially could raises the complexity of the analysis.

4. Approach:

Strengths

- Well-developed modeling techniques linked to experimental technique designed to model and identify IgG Immune complexes and link those to function.
- Clear communication between the computational and experimental parts of the study, which will allow modification of models should the need arise and in parallel the design of new experiments if those are suggested by the modeling.
- If successful this approach should lead to a much greater understanding of both the landscape of IgG IC types and their functional importance allowing for more careful design of these for clinical purposes.

Weaknesses

- Attempts to link the murine results to human results. In aim 2, the linking between murine and human data depends on the assumption that similar patterns will be found. Aim 3 is also affected by this assumption, if to a lesser extent. Aim 3 also assumes that the humanized mouse will be representative of human immune responses but still generate comparable phenomena. However, it will be difficult to distinguish if the resemblance is due to more murine parts of the humanized mouse or due to human parts.
- A minor weakness is the use of Pearson Correlation. Since IgG expression may not be normally distributed, a non-parametric Spearman test would be better method for the analysis.

5. Environment:

Strengths

- Both institutions and labs are well suited to the computational and experimental tasks that are suggested in the application.

Weaknesses

- None noted.

CRITIQUE 3

Significance:	2
Investigator(s):	1
Innovation:	2
Approach:	2
Environment:	1

Overall Impact: The central hypothesis described in the project summary of this proposal is that “two IgGs can elicit distinct responses when present in combination from what would be suggested by the response to either on its own.” To investigate this hypothesis a computational model will be developed that will be capable of predicting FcR binding for multivalent immune complexes made up of multiple types of IgGs. This model will be verified by in vitro studies such as cytokine secretion and phagocytosis of fluorescently labeled immune complexes. To correlate human and murine effector responses, a homology map of human and murine IgG isotypes based on effector cell responses will be produced. Mouse models of IgG dependent cell killing, immunothrombocytopenia and cytotoxic antibody-mediated B cell depletion, will then be used to identify synergistic antibody combinations and to verify combinations predicted by the models. Experiments will be conducted to identify the effector cells responsible for the synergistic cell killing. The goal of these experiments will be to provide insight for designing therapeutic antibodies or antibody combinations with efficient effector cell responses, and to better understand antibody dependent immune responses.

1. Significance:

Strengths

- This project is of high significance in that it addresses the prediction of Fc-mediated effector function using modern mathematical models based on multivalent interactions of immune complexes with Fc-gamma receptors. There is a wealth of older modeling literature, largely based on multivalent interactions of antigen and BCR or cross-linking of the Fc-epsilon receptor by immune complexes. However, there is little in the way of contemporary modeling of the interactions of immune complexes and Fc-gamma receptors.
- The proposed studies, if successful, will greatly inform the ability to predict in vivo mechanisms of ADCC and ADCP.
- The methods developed in this application will provide a generalized approach to predicting Fc-gamma receptor mediated effector function across systems and species.

Weaknesses

- None were noted.

2. Investigators:

Strengths

- The principal investigator is an outstanding young scientist in the early phase of his career. He is well-trained and highly productive working at the important interface of “wet” biology and computational biology.
- Another strength of the application is the involvement of Dr. Falk Nimmerjhan, who is an internationally recognized authority of Fc-mediated effector function.
- The collaboration between Drs. Meyer and Nimmerjhan resulted in a seminal paper published in Cell Systems describing the development and parameterization of the basic model used in this application. The investigators have an outstanding collaboration.

Weaknesses

- None were noted.

3. Innovation:

Strengths

- The project is highly innovative in that it is built upon a unique model to predict the strength of Fc-mediated effector function. This model extends and supersedes the multivalent aggregation models from earlier studies of the Fc-epsilon receptor. At this point, this model is unique in the field of Fc-mediated effector function.
- If successful, this project will provide significant impact on understanding the mechanisms of Fc-gamma mediated effector function that likely extends to clinical applications of the mathematical model.

Weaknesses

- None were noted.

4. Approach:

Strengths

- The overall strategy is built upon a new model of multivalent cross-linking of Fc-gamma receptors that predicts biological efficacy. The basic model was published in Cell Systems, 2018 by Drs. Meyer and Nimmerjhan.
- Aim 1 is strong in that it will extend the basic model to mixed IgG compositions that are likely to be representative of polyclonal antibody responses in vivo. The logic and tools proposed for this aim are highly appropriate and likely to yield new information. The investigators have carefully considered caveats and alternative solutions to problems.
- Aim 2 is especially strong as it proposes a unique mapping of IgG subclass functions species. The differences between murine and human IgG subclasses and Fc-gamma receptors are well known and it was not possible to construct a 1:1 structure function map of Fc-gamma mediated effector function. The investigators rightfully recognize that despite the differences in IgG subclasses and Fc-gamma receptors between mice and humans both humans use combinations of the two sets of molecules to solve the same problem. The investigator’s approach is to ferret out the interspecies conservation of function between the two species using tensor decomposition to map IC composition and effector responses.
- Aim 3 is also strong in that it will extend the model to identify and predict synergy among Fc-gamma receptors and in vivo functions.

Weaknesses

- None were noted.

5. Environment:

Strengths

- The environment is outstanding.

Weaknesses

- None were noted.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: NOT APPLICABLE (CODE 10)

INCLUSION OF WOMEN PLAN: NOT APPLICABLE

INCLUSION OF MINORITIES PLAN: NOT APPLICABLE

INCLUSION OF INDIVIDUALS ACROSS THE LIFE SPAN: NOT APPLICABLE

VERTEBRATE ANIMAL: ACCEPTABLE (CODE 30)
The protection of Vertebrate Animal welfare is adequately described.

BIOHAZARDS COMMENT: ACCEPTABLE
The plan to prevent risks during handling of biohazard materials or samples is adequate.

FOREIGN INSTITUTION: NOT APPLICABLE

SELECT AGENTS: NOT APPLICABLE

RESOURCE SHARING PLANS

DATA SHARING PLANS: ACCEPTABLE
There is an adequate plan for data sharing outlined in the application.
MODEL ORGANISM SHARING PLANS: NOT APPLICABLE
GENOMIC DATA SHARING PLAN: NOT APPLICABLE

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES: ACCEPTABLE
Adequate plans for authentication of key resources are outlined in the application.

BUDGETARY OVERLAP:
No budgetary overlap was noted.

COMMITTEE BUDGET RECOMMENDATIONS:
The budget was recommended as requested for all years.

Footnotes for 1 U01 AI148119-01; PI Name: Meyer, Aaron Samuel

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.