

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                  |                                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------|
| PI: <b>Meyer, Aaron Samuel</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Title: Mapping the effector response space of antibody combinations                              |                                                    |
| Received: 02/01/2019                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | FOA: AI18-042                                                                                    | Council: 10/2019                                   |
| Competition ID: FORMS-E                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | FOA Title: Fc-Dependent Mechanisms of Antibody-Mediated Killing (U01 Clinical Trial Not Allowed) |                                                    |
| <b>1 U01 AI148119-01</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Dual:                                                                                            | Accession Number: 4259985                          |
| IPF: 577505                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Organization: UNIVERSITY OF CALIFORNIA LOS ANGELES                                               |                                                    |
| Former Number:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Department: Bioengineering                                                                       |                                                    |
| IRG/SRG: ZAI1 SB-I (S1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | AIDS: N                                                                                          | Expedited: N                                       |
| Subtotal Direct Costs<br>(excludes consortium F&A)<br>Year 1: <br>Year 2: <br>Year 3: <br>Year 4: <br>Year 5:  | Animals: Y<br>Humans: N<br>Clinical Trial: N<br>Current HS Code: 10<br>HESC: N                   | New Investigator: N<br>Early Stage Investigator: N |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                  |                                                    |
| <i>Senior/Key Personnel:</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <i>Organization:</i>                                                                             | <i>Role Category:</i>                              |
| Aaron Meyer                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | The Regents of the University of California, Los Angeles                                         | PD/PI                                              |
| Falk Nimmerjahn                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Friedrich-Alexander University Erlangen-Nuremberg                                                | MPI                                                |

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APPLICATION FOR FEDERAL ASSISTANCE  
**SF 424 (R&R)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                                                                                                                                                                                                     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>3. DATE RECEIVED BY STATE</b>                                                                                                                                                                                                                                                                                                                                                                                                                                |                               | <b>State Application Identifier</b>                                                                                                                                                                                 |
| <b>1. TYPE OF SUBMISSION*</b>                                                                                                                                                                                                                                                                                                                                                                                                                                   |                               | <b>4.a. Federal Identifier</b>                                                                                                                                                                                      |
| <input type="radio"/> Pre-application <input type="radio"/> Application <input checked="" type="radio"/> Changed/Corrected Application                                                                                                                                                                                                                                                                                                                          |                               | <b>b. Agency Routing Number</b>                                                                                                                                                                                     |
| <b>2. DATE SUBMITTED</b><br>2019-02-01                                                                                                                                                                                                                                                                                                                                                                                                                          | <b>Application Identifier</b> | <b>c. Previous Grants.gov Tracking Number</b><br>GRANT12778592                                                                                                                                                      |
| <b>5. APPLICANT INFORMATION</b> <span style="float: right;"><b>Organizational DUNS*</b> [REDACTED]</span>                                                                                                                                                                                                                                                                                                                                                       |                               |                                                                                                                                                                                                                     |
| Legal Name*: The Regents of the University of California, Los Angeles<br>Department:<br>Division:<br>Street1*: Office of Contract and Grant Administration<br>Street2*: [REDACTED]<br>City*: Los Angeles<br>County: Los Angeles County<br>State*: CA: California<br>Province:<br>Country*: USA: UNITED STATES<br>ZIP / Postal Code*: 90095-1406                                                                                                                 |                               |                                                                                                                                                                                                                     |
| Person to be contacted on matters involving this application<br>Prefix: Mr.    First Name*: Evan    Middle Name:    Last Name*: Garcia    Suffix:<br>Position/Title: Grant Officer<br>Street1*: [REDACTED]<br>Street2:<br>City*: Los Angeles<br>County: Los Angeles County<br>State*: CA: California<br>Province:<br>Country*: USA: UNITED STATES<br>ZIP / Postal Code*: 90095-1406<br>Phone Number*: [REDACTED]    Fax Number: [REDACTED]    Email: [REDACTED] |                               |                                                                                                                                                                                                                     |
| <b>6. EMPLOYER IDENTIFICATION NUMBER (EIN) or (TIN)*</b> [REDACTED]                                                                                                                                                                                                                                                                                                                                                                                             |                               |                                                                                                                                                                                                                     |
| <b>7. TYPE OF APPLICANT*</b> <span style="float: right;">H: Public/State Controlled Institution of Higher Education</span>                                                                                                                                                                                                                                                                                                                                      |                               |                                                                                                                                                                                                                     |
| Other (Specify):<br><b>Small Business Organization Type</b> <input type="radio"/> Women Owned <input type="radio"/> Socially and Economically Disadvantaged                                                                                                                                                                                                                                                                                                     |                               |                                                                                                                                                                                                                     |
| <b>8. TYPE OF APPLICATION*</b>                                                                                                                                                                                                                                                                                                                                                                                                                                  |                               | If Revision, mark appropriate box(es).                                                                                                                                                                              |
| <input checked="" type="radio"/> New <input type="radio"/> Resubmission<br><input type="radio"/> Renewal <input type="radio"/> Continuation <input type="radio"/> Revision                                                                                                                                                                                                                                                                                      |                               | <input type="radio"/> A. Increase Award <input type="radio"/> B. Decrease Award <input type="radio"/> C. Increase Duration<br><input type="radio"/> D. Decrease Duration <input type="radio"/> E. Other (specify) : |
| <b>Is this application being submitted to other agencies?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No    What other Agencies?                                                                                                                                                                                                                                                                                                            |                               |                                                                                                                                                                                                                     |
| <b>9. NAME OF FEDERAL AGENCY*</b><br>National Institutes of Health                                                                                                                                                                                                                                                                                                                                                                                              |                               | <b>10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER</b><br>TITLE:                                                                                                                                                  |
| <b>11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*</b><br>Mapping the effector response space of antibody combinations                                                                                                                                                                                                                                                                                                                                            |                               |                                                                                                                                                                                                                     |
| <b>12. PROPOSED PROJECT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                     |                               | <b>13. CONGRESSIONAL DISTRICTS OF APPLICANT</b>                                                                                                                                                                     |
| Start Date*    Ending Date*<br>12/01/2019    11/30/2024                                                                                                                                                                                                                                                                                                                                                                                                         |                               | CA-033                                                                                                                                                                                                              |

**SF 424 (R&R)** APPLICATION FOR FEDERAL ASSISTANCE**Page 2****14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: First Name\*: Aaron Middle Name: Last Name\*: Meyer Suffix:

Position/Title: Assistant Professor

Organization Name\*: The Regents of the University of California, Los Angeles

Department: Bioengineering

Division:

Street1\*: [REDACTED]

Street2\*: [REDACTED]

City\*: Los Angeles

County: Los Angeles County

State\*: CA: California

Province:

Country\*: USA: UNITED STATES

ZIP / Postal Code\*: 90095-1406

Phone Number\*: [REDACTED] Fax Number: [REDACTED] Email\*: [REDACTED]

**15. ESTIMATED PROJECT FUNDING**

- a. Total Federal Funds Requested\* [REDACTED]
- b. Total Non-Federal Funds\* [REDACTED]
- c. Total Federal & Non-Federal Funds\* [REDACTED]
- d. Estimated Program Income\* [REDACTED]

**16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?\***

- a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
- DATE:
- b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR ☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

**17. By signing this application, I certify (1) to the statements contained in the list of certifications\* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances \* and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)**

☒ I agree\*

\* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

**18. SFLLL or OTHER EXPLANATORY DOCUMENTATION**

File Name:

**19. AUTHORIZED REPRESENTATIVE**

Prefix: Mr. First Name\*: Evan Middle Name: Last Name\*: Garcia Suffix:

Position/Title\*: Grant Officer

Organization Name\*: The Regents of the University of California, Los Angeles

Department: Office of Contract & Grant Adm

Division:

Street1\*: [REDACTED]

Street2\*: [REDACTED]

City\*: Los Angeles

County: Los Angeles County

State\*: CA: California

Province:

Country\*: USA: UNITED STATES

ZIP / Postal Code\*: 90095-1406

Phone Number\*: [REDACTED] Fax Number: [REDACTED] Email\*: [REDACTED]

Signature of Authorized Representative\*

Date Signed\*

02/01/2019

**20. PRE-APPLICATION** File Name:**21. COVER LETTER ATTACHMENT** File Name: 20190201\_U01\_CoverLtr1053974464.pdf

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**Project/Performance Site Location(s)****Project/Performance Site Primary Location**

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Regents of the University of California, Los Angeles  
Duns Number: [REDACTED]  
Street1\*: [REDACTED]  
Street2\*: [REDACTED]  
City\*: Los Angeles  
County: Los Angeles  
State\*: CA: California  
Province:  
Country\*: USA: UNITED STATES  
Zip / Postal Code\*: 90095-1600  
Project/Performance Site Congressional District\*: CA-033

---

**Project/Performance Site Location 1**

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Friedrich-Alexander University Erlangen-Nurember  
DUNS Number: [REDACTED]  
Street1\*: [REDACTED]  
Street2\*: [REDACTED]  
City\*: Erlangen  
County:  
State\*:  
Province:  
Country\*: DEU: GERMANY  
Zip / Postal Code\*: 91058-0000  
Project/Performance Site Congressional District\*: 00-000

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**Additional Location(s)**

File Name:

## RESEARCH &amp; RELATED Other Project Information

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                           |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| <b>1. Are Human Subjects Involved?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No                                                                                                                                                                                                                                                                                                                                                                                                   |                                           |
| 1.a. If YES to Human Subjects<br>Is the Project Exempt from Federal regulations? <input type="radio"/> Yes <input type="radio"/> No<br>If YES, check appropriate exemption number:   1 <input type="text"/> 2 <input type="text"/> 3 <input type="text"/> 4 <input type="text"/> 5 <input type="text"/> 6 <input type="text"/> 7 <input type="text"/> 8 If<br>NO, is the IRB review Pending? <input type="radio"/> Yes <input type="radio"/> No<br>IRB Approval Date:<br>Human Subject Assurance Number |                                           |
| <b>2. Are Vertebrate Animals Used?*</b> <input checked="" type="radio"/> Yes <input type="radio"/> No                                                                                                                                                                                                                                                                                                                                                                                                   |                                           |
| 2.a. If YES to Vertebrate Animals<br>Is the IACUC review Pending? <input checked="" type="radio"/> Yes <input type="radio"/> No<br>IACUC Approval Date:<br>Animal Welfare Assurance Number <input type="text"/>                                                                                                                                                                                                                                                                                         |                                           |
| <b>3. Is proprietary/privileged information included in the application?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No                                                                                                                                                                                                                                                                                                                                                             |                                           |
| <b>4.a. Does this project have an actual or potential impact - positive or negative - on the environment?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No                                                                                                                                                                                                                                                                                                                            |                                           |
| 4.b. If yes, please explain:<br>4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? <input type="radio"/> Yes <input type="radio"/> No<br>4.d. If yes, please explain:                                                                                                                                                                              |                                           |
| <b>5. Is the research performance site designated, or eligible to be designated, as a historic place?*</b> <input type="radio"/> Yes <input checked="" type="radio"/> No                                                                                                                                                                                                                                                                                                                                |                                           |
| 5.a. If yes, please explain:                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                           |
| <b>6. Does this project involve activities outside the United States or partnership with international collaborators?*</b> <input checked="" type="radio"/> Yes <input type="radio"/> No                                                                                                                                                                                                                                                                                                                |                                           |
| 6.a. If yes, identify countries:   Germany                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                           |
| 6.b. Optional Explanation:                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                           |
| <b>7. Project Summary/Abstract*</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Filename<br>project_summary1053974458.pdf |
| <b>8. Project Narrative*</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | project_narrative1053974455.pdf           |
| <b>9. Bibliography &amp; References Cited</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Bibliography1053818961.pdf                |
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| <b>11. Equipment</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | MajorEquipment1053974639.pdf              |
| <b>12. Other Attachments</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Foreign_Justification1053818959.pdf       |

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.

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.



## FACILITIES & OTHER RESOURCES – UNIVERSITY OF CALIFORNIA, LOS ANGELES

**Laboratory:** Dr. Meyer's lab occupies dedicated laboratory space [REDACTED] Engineering V (EV). EV opened in Fall of 2007 and provides a state-of-the-art research home for the Department of Bioengineering faculty members and their labs. Dr. Meyer is allotted over 800 square feet of space including laboratory and shared research support space. A portion of this (200 sq ft) is a dedicated, positive-pressure cell culture room. This space includes access to air/gas and vacuum connections at both the bench and hood locations, internal deionized water systems, and appropriately scaled HVAC and electrical service. Recent renovations (2017) have ensured it is specifically tailored to the work proposed here. The lab space is within close proximity to shared resources, conference room space, and office space.

**Computational:** Dr. Meyer has two computers: (1) a Mac Pro located in his office (Mac OS), and (2) a MacBook Pro laptop (Mac OS). Each technician and student is equipped with a similar laptop. The University provides a subscription to Zoom Meeting telecommunication software for remote meetings and provides all campus members with unlimited storage versioned and stored off-site through the Box service. The engineering school provides all faculty, students, and staff with access to software development tools, office software, and statistical software for common and specialized needs (e.g. MATLAB). The Meyer lab maintains two high-performance, 32-core servers for computationally intensive jobs. For long-term storage, the lab maintains a network attached storage server (Synology) with 4TB of space. In addition, the Hoffman2 cluster on campus provides support for larger computational tasks with 13,340 cores and over 50TB of memory. All computers in the Meyer lab are automatically and continually backed up to off-site storage. The combination of these information technologies contributes to efficient data handling and optimal communication among members of the research team.

**Office:** As a faculty member of the Bioengineering Department within the Samueli School Of Engineering, Dr. Meyer has an office one floor away from his laboratory. It is equipped with desk, task chairs, two 4-drawer filing cabinets, and hardwired high-speed access. His lab members have access to dedicated, separate office space in the lab's own room for writing and study. The students'/ technician's shared office space has similar access and is equipped with four individual desks, four task chairs, and five 2-drawer filing cabinets. There is also access to the internet through the University's wireless network. These facilities ensure that Dr. Meyer and his immediate research team will have the necessary space in which to formulate experiments, analyze results, and prepare manuscripts for publication.

**Institutional Support:** Full administrative support is provided by the engineering school. This includes staff individually dedicated to funds management, student advisement, purchasing, and facilities maintenance.

**Intellectual Rapport:** The academic structure is founded on an interdisciplinary ethic. There are many opportunities to meet, hear about, and discuss research, including the Bioengineering seminar series, the Bioinformatics seminar series, the Stem Cell Center meetings, Jonsson Comprehensive Cancer Center speaker series and discussion meetings, and the QCBio (Institute for Quantitative and Computational Biology) seminar series. The Meyer lab additionally participates in a regular joint meeting with the labs of Alex Hoffman (Immunology) and Roy Wollman (Biological Chemistry) on modeling and microscopy.

All members of the Meyer lab receive regular feedback and career planning formalized through individualized development plans and annual reviews. Additionally, graduate students are assigned a thesis committee that helps to provide independent mentorship and career planning advice.

### Shared Resources:

As a member of the Jonsson Cancer Center and the Broad Stem Cell Center, Dr. Meyer has access to extensive resources within the core facilities including flow cytometry, genomics, molecular screening, small animal imaging, translational pathology, high-throughput sequencing, imaging, and biostatistics. These services are available to members of the centers at a subsidized rate. Particularly relevant to the work

proposed here:

The Advanced Light Microscopy/ Spectroscopy Core is housed within the California NanoSystems Institute and provides consultation, services, and support for the application of novel spectroscopic methods and advanced image analysis techniques for the study of macromolecules, cellular dynamics and nanoscale characterization of bio-materials.

The Janis V. Giorgi Flow Cytometry Core Laboratory is a part of the UCLA medical school and offers consultation, services, and support for flow analysis and sorting. As a member of the Jonsson Comprehensive Cancer Center, the Meyer lab receives a discounted rate on all services.

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## Facility and RESOURCES

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Laboratory:

Dr. Nimmerjahn has a ~4,000 sq. ft. laboratory at the Institute of Genetics at the Friedrich-Alexander University Erlangen-Nuremberg (FAU), as well as in house core facilities for FACS analysis, cell sorting and mouse housing. The spaces include three wet labs, tissue culture facilities, cold room, imaging laboratory, and office space for all staff.

Clinical: N/A

Biocontainment: N/A. All work done at the Institute of Genetics will involve recombinant proteins and tissue derived from mice. The Nimmerjahn laboratory is equipped for BSL-1 and -2 studies for lentiviral infection of human cells and maintenance of human BSL2 cell lines.

Animal:

The Institute of Genetics has a 6000sq. ft. animal facility to house up to 6000 animals in IVC cages or under full barrier conditions. The animal facility is maintained by professional animal technicians and also contains procedure rooms to bleed mice and recover mouse organs.

Computer:

The laboratory has ~10 high-end Macintosh and windows workstations. For additional data processing, we have access to the supercomputing cluster of the technical faculty at the University.

Office:

The P.I. and administrative staff have enclosed office space. Two half-time administrative assistants provide administrative support.

Other:

Other close collaborations and interchanges exist with > 30 immunology and 15 molecular biology faculty at FAU with expertise in human and mouse immunology (incl. cancer immunology, neuroimmunology, cancer immunology), cell biology (stem cell biology, cancer biology, development, virology, microbiology), x-ray crystallography and electron microscopy.

SCIENTIFIC ENVIRONMENT: Contribution to the probability of success.

The laboratory of Dr. Nimmerjahn is able to produce and purify antibodies and recombinant proteins at large scale, perform functional tests in tissue culture and in animal models systems. The laboratory houses two refrigerated shakers, four tissue culture incubators, four biological hoods, microscopes, 3 PCR machines, cold room, water purifier, fume hood, five -86°C and five -20°C freezers, and an automatic refill liquid nitrogen storage freezer. For protein purification and analysis, the laboratory has two ATKApure and FPLC systems, multiple electrophoresis and blotting apparatuses, low and high speed chromatography pumps, a UV/Vis spectrophotometer, a UV/kinetics plate reader, and low speed and high speed Beckman centrifuges. A BiaCore to measure binding affinities and kinetics is shared with the Department of Chemistry at the FAU.

SPECIAL FACILITIES: Describe facilities used for working with biohazard, or other dangerous substances. N/A

## **EQUIPMENT - UNIVERSITY OF CALIFORNIA, LOS ANGELES**

Meyer Laboratory — The laboratory is equipped all necessary equipment for performing the proposed work, including:

- Multiple electrophoresis units
- Thermocycler
- Swing-bucket centrifuge
- Fixed-angle high-speed centrifuge
- Two microfuges
- Two bacterial shakers and incubators
- Photographic equipment for documentation of gels and microscopy
- Refrigerators
- Standard freezers
- One  $-80^{\circ}\text{C}$  freezer
- NanoDrop spectrophotometer

Cell culture facilities (biological safety cabinets, incubators, microscopes, assorted other equipment) are all contained in a tissue culture room exclusive to the Meyer lab. Included in the Meyer lab's cell culture facility in one of the Meyer lab incubators is an Incucyte Zoom live-cell imaging system. This system provides real-time monitoring of cells in culture and is equipped with software and hardware for automated cell migration, proliferation, and apoptosis assays.

In addition, there is extensive shared equipment and services available across the UCLA campus. As a member of the Jonsson Cancer Center and the Broad Stem Cell Center, Dr. Meyer has access to extensive resources within the core facilities including flow cytometry, genomics, molecular screening, small animal imaging, translational pathology, high-throughput sequencing, imaging, and biostatistics. These services are available to members of the centers at a subsidized rate.

### **Common Equipment**

- Dynamic light scattering analyzer
- Backup freezers
- Milli-Q water
- Ice machine
- Spectrophotometer
- Plate reader: 96 & 384 well, Multimode (UV/VIS/IR/Luminescence/Fluorescence)
- Centrifuges
- Autoclave

### **Shared Core Equipment**

#### **Advanced Light Microscopy/ Spectroscopy Core**

The Advanced Light Microscopy/ Spectroscopy Core is housed within the California NanoSystems Institute and provides consultation, services and support for the application of novel spectroscopic methods and advanced image analysis techniques for the study of macromolecules, cellular dynamics and nanoscale characterization of bio-materials.

- Confocal SP8-SMD

- Confocal SP8 Compact
- Confocal SP5 Blue
- Confocal SP5 STED
- Confocal SP2 MP-FLIM
- Confocal microALEX-FCS
- Wide-Field CCD Microinjection Inverted
- Wide-Field NUANCE Microinjection Upright
- Wide-Field CCD IN SITU Upright
- *In vivo* Maestro 2
- Leica LMD7000
- Leica RM2235 Rotary Microtome
- Leica M205 FA Fluorescence Stereomicroscope
- Dissection Microscope and Microinjector
- DMIL Microscope with SPOT Camera
- SP8 Analysis Workstation

## Flow Cytometry Core

The Janis V. Giorgi Flow Cytometry Core Laboratory is a part of the UCLA medical school and offers consultation, services and support for flow analysis and sorting. As a member of the Jonsson Comprehensive Cancer Center the Meyer lab receives a discounted rate on all services.

- LSRFortessa X-20 SORP
- SORP BD LSRII Analytic Flow Cytometer
- SORP BD HTLSRII Analytic Flow Cytometer
- SORP BD LSRII (IMED) Analytic Flow Cytometer
- ImageStreamx MarkII Imaging Flow Cytometer
- Helios (a CyTOF system) Mass Cytometer
- FACSAriaIII High-Speed Cell Sorter contained in a BioProtect IV class II biosafety cabinet
- FACSAria I (II) High-Speed Cell Sorter
- SORP FACSAriaII High-Speed Cell Sorter
- RoboSep Magnetic Cell Separator

## Information Technology

- The engineering school provides all faculty, students, and staff with access to software development tools, office software, and statistical software for common and specialized needs (e.g. MATLAB).
- UCLA provides all campus members with unlimited storage versioned and stored off-site through the Box service.
- The Meyer lab maintains two high-performance, 32-core servers for computationally intensive jobs. In addition, the Hoffman2 cluster on campus provides support for larger computational tasks with 13,340 cores and over 50TB of memory.
- All computers in the Meyer lab are automatically and continually backed up to off-site storage.

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## EQUIPMENT RESOURCES

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### **The Institute of Genetics at the Friedrich-Alexander University Erlangen-Nuremberg: Falk Nimmerjahn**

The Institute of Genetics is equipped with state of the art laboratories and animal housing facilities for performing immunological and molecular biology research at the highest standard. The Insitute has three different FACS analyzers and one BD FACS Aria III cell sorter to isolate cell populations from mouse and human blood and different mouse organs. Furthermore an ADVIA Heamtology system (Siemens) allows to determine cellular changes in mouse and human blood and serum samples without the need for staining with fluorescently labeled antibodies. For histology, standard cryotomes and microtomes are available to perform immunohistochemical and immunofluorescent analysis. Biosafety level I and II tissue culture facilities are available to study mouse and human cells of patients. Moreover, HPLC and FPLC machines for antibody characterization and purification are available. The animal facility can house up to 6000 mice in a full barrier or IVC facility.

## Foreign Site Justification

Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and the Nimmerjahn lab specifically are included in this proposal because they bring unique and vital, internationally-recognized expertise in the immunology of effector cell responses to IgG antibodies. Prof. Nimmerjahn's expertise extends to the experimental methods and reagents used throughout both Aim 1 and 3, the *in vivo* models used to validate the computational predictions within the project, and input regarding integration of the computational and experimental efforts. His participation is essential to effective execution of this project and will help to ensure the success of this proposal's important goal understanding how antibodies operate to direct target cell killing when present in combination. By accomplishing this goal, including FAU furthers the goals of the NIH more effectively than domestic alternatives.

## RESEARCH &amp; RELATED Senior/Key Person Profile (Expanded)

| PROFILE - Project Director/Principal Investigator |                                                          |                               |                   |
|---------------------------------------------------|----------------------------------------------------------|-------------------------------|-------------------|
| Prefix:                                           | First Name*: Aaron                                       | Middle Name                   | Last Name*: Meyer |
| Suffix:                                           |                                                          |                               |                   |
| Position/Title*:                                  | Assistant Professor                                      |                               |                   |
| Organization Name*:                               | The Regents of the University of California, Los Angeles |                               |                   |
| Department:                                       | Bioengineering                                           |                               |                   |
| Division:                                         |                                                          |                               |                   |
| Street1*:                                         |                                                          |                               |                   |
| Street2:                                          |                                                          |                               |                   |
| City*:                                            | Los Angeles                                              |                               |                   |
| County:                                           | Los Angeles County                                       |                               |                   |
| State*:                                           | CA: California                                           |                               |                   |
| Province:                                         |                                                          |                               |                   |
| Country*:                                         | USA: UNITED STATES                                       |                               |                   |
| Zip / Postal Code*:                               | 90095-1406                                               |                               |                   |
| Phone Number*:                                    |                                                          | Fax Number:                   |                   |
| E-Mail*:                                          |                                                          |                               |                   |
| Credential, e.g., agency login:                   |                                                          |                               |                   |
| Project Role*: PD/PI                              | Other Project Role Category:                             |                               |                   |
| Degree Type:                                      | Degree Year:                                             |                               |                   |
| Attach Biographical Sketch*:                      | File Name:                                               | biosketch_meyer1053818960.pdf |                   |
| Attach Current & Pending Support:                 | File Name:                                               |                               |                   |



| PROFILE - Senior/Key Person              |                                                                                   |                                         |                        |         |
|------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------|------------------------|---------|
| Prefix: Dr.                              | First Name*: Falk                                                                 | Middle Name                             | Last Name*: Nimmerjahn | Suffix: |
| Position/Title*:                         | Professor - Chair of Genetics                                                     |                                         |                        |         |
| Organization Name*:                      | Friedrich-Alexander University Erlangen-Nuremberg                                 |                                         |                        |         |
| Department:                              | Biology                                                                           |                                         |                        |         |
| Division:                                |                                                                                   |                                         |                        |         |
| Street1*:                                |  |                                         |                        |         |
| Street2:                                 |                                                                                   |                                         |                        |         |
| City*:                                   | Erlangen                                                                          |                                         |                        |         |
| County:                                  |                                                                                   |                                         |                        |         |
| State*:                                  |                                                                                   |                                         |                        |         |
| Province:                                |                                                                                   |                                         |                        |         |
| Country*:                                | DEU: GERMANY                                                                      |                                         |                        |         |
| Zip / Postal Code*:                      | 91058-0000                                                                        |                                         |                        |         |
| Phone Number*                            |  |                                         | Fax Number:            |         |
| E-Mail*:                                 |  |                                         |                        |         |
| Credential, e.g., agency login: FNIMMERJ |                                                                                   |                                         |                        |         |
| Project Role*: PD/PI                     |                                                                                   | Other Project Role Category:            |                        |         |
| Degree Type:                             |                                                                                   | Degree Year:                            |                        |         |
| Attach Biographical Sketch*:             | File Name:                                                                        | Biosketch_Nimmerjahn_20191054050156.pdf |                        |         |
| Attach Current & Pending Support:        |                                                                                   | File Name:                              |                        |         |

**BIOGRAPHICAL SKETCH**

NAME: Aaron Samuel Meyer

eRA COMMONS USER NAME: [REDACTED]

POSITION TITLE: Assistant Professor of Bioengineering

**EDUCATION/TRAINING**

| INSTITUTION AND LOCATION                     | DEGREE | Completion Date | FIELD OF STUDY         |
|----------------------------------------------|--------|-----------------|------------------------|
| University of California, Los Angeles (UCLA) | B.S.   | 6/2009          | Bioengineering         |
| Massachusetts Institute of Technology (MIT)  | Ph.D.  | 6/2014          | Biological Engineering |

**A. Personal Statement**

I have a background in biological engineering, with training in applied machine learning, cell signaling, and cancer. My research broadly applies integrated experimental and theoretical approaches to understanding the complex signaling that underlies tumor-immune communication and resistance to therapies. My lab is particularly interested in how cues operate in combination within the tumor microenvironment to direct cancer and immune effector cell behavior and how we might optimally target dysregulation. For example, my previous work has shown how (1) IgG antibody effector function is transduced through multiple receptors and effector cells, (2) an RTK family expressed in cancer and innate immune cells is activated in response to extracellular cues in the tumor microenvironment, (3) bypass resistance to therapy operates through coordinated pathway activation, and (4) receptor crosstalk is a mechanism of signal diversification promoting breast tumor invasion.

I have extensive experience with collaborative projects involving multiple labs such as in the work proposed here and have a track record of success mentoring graduate students, postdoctoral associates, and technical associates.

1. Robinett, R.A., N. Guan, A. Lux, M. Biburger, F. Nimmerjahn, **A.S. Meyer**. (2018). "Dissecting FcγR Regulation Through a Multivalent Binding Model." *Cell Systems*. 2018 July 25; 6(7): 1–8.
2. **Meyer, A.S.<sup>†</sup>**, A.J.M. Zweemer, D.A. Lauffenburger<sup>†</sup>. (2015). The AXL Receptor Is a Sensor of Ligand Spatial Heterogeneity. *Cell Systems*, 1(1):25–36. PMCID: 4520549.
3. Manole, S., E.J. Richards, **A.S. Meyer**. (2016). JNK pathway activation modulates acquired resistance to EGFR/HER2 targeted therapies. *Cancer Research*. Sept 15; 76 (18): 5219-5228. PMCID: 5026573.
4. **Meyer, A.S.**, M.A. Miller, F.B. Gertler, D.A. Lauffenburger. (2013). The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. *Science Signaling*, 6(287), ra66. PMCID: 3947921.

<sup>†</sup>Co-corresponding authors.

**B. Positions and Honors****Positions and Employment**

**2006–2009** Undergraduate Researcher, Bioengineering Department, UCLA

**2008** Summer intern, Bioprocess Development Division, Schering-Plough Corporation, Watchung, NJ

**2009–2014** Graduate Researcher, Department of Biological Engineering, MIT

**2014–2017** Principal Investigator/Research Fellow, Koch Institute for Integrative Cancer Research, MIT

**2017–Present** Assistant Professor, Bioengineering Department, UCLA

## Other Experience and Professional Memberships

**2010–2012** Member, MIT Biological Engineering Retreat Organizing Committee

**2010–2013** Coordinator, MIT Biological Engineering Graduate Student Board

**2010–Present** Member, Biomedical Engineering Society

**2014–2017** Committee Member, Association of Early Career Cancer Systems Biologists

**2015–Present** Organizer, Systems Approaches to Cancer Biology meeting

**2017–Present** Chair, Association of Early Career Cancer Systems Biologists

## Honors

**2009** Momen Presidential Fellowship, MIT

**2009** Graduate Research Fellowship, National Science Foundation

**2010** Breast Cancer Research Predoctoral Fellowship, Department of Defense

**2012** Repligen Fellowship in Cancer Research, Koch Institute

**2012** Travel grant to attend PTMs in Cell Signaling Conference in Copenhagen, Denmark

**2013** Whitaker Fellowship, MIT

**2013** Siebel Scholar, Class of 2014

**2016** Ten to Watch, Amgen Scholars Foundation

**2016–2017** Fellowship, Terri Brodeur Breast Cancer Foundation

## C. Contribution to Science

### Understanding and targeting receptor family communication

Often receptor families have many ligands and receptors while being expressed across many cell populations. This multi-layered multiplicity confounds intuition about how these receptors are regulated in homeostasis, dysregulated in disease, and might be targeted with therapies. Through a combination of modeling and experiment, we have been working to develop the tools enabling improved understanding of these families' regulation and how best to target them. Within the FcγR family, which enacts effector function in response to IgG antibodies, we recently showed that a multivalent binding model could predict effector function *in vivo* better than previously-used metrics, which will enable more potent anti-tumor antibodies (1). Within the TAM family of receptor tyrosine kinases, we identified that AXL can be transactivated from ErbB receptors and that this transactivation drives the invasiveness of breast carcinoma cells more so than the signaling from the ErbB receptors themselves (3). We then developed a kinetic model of AXL activation, mechanistically explaining the dependence of the receptor upon phosphatidylserine for activation (2, 4). This basic understanding of TAM and FcγR signaling enables more rationally designed therapies and understanding of which factors in the tumor microenvironment drive activation.

1. Robinett, R.A., N. Guan, A. Lux, M. Biburger, F. Nimmerjahn, **A.S. Meyer**. (2018). "Dissecting FcγR Regulation Through a Multivalent Binding Model." *Cell Systems*. 2018 July 25; 6(7): 1–8.
2. **Meyer, A.S.<sup>†</sup>**, A.J.M. Zweemer, D.A. Lauffenburger<sup>†</sup>. (2015). The AXL Receptor Is a Sensor of Ligand Spatial Heterogeneity. *Cell Systems*, 1(1):25–36. PMID: 4520549.

3. **Meyer, A.S.**, M.A. Miller, F.B. Gertler, D.A. Lauffenburger. (2013). The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. *Science Signaling*, 6(287), ra66. PMCID: 3947921.
4. Zweemer, A., C.B. French, J. Mesfin, S. Gordonov, **A.S. Meyer**, and D.A. Lauffenburger. (2017). Apoptotic Bodies Elicit Gas6-mediated Migration of AXL-expressing Tumor Cells. *Molecular Cancer Research*. 2017 Dec; 15(12): 1656–1666. PMCID: 5957761.

<sup>†</sup>Co-corresponding authors.

## Therapeutic resistance and design

The benefits cancer patients derive from targeted therapies are limited by genetic and non-genetic mechanisms of resistance. This is in part due to an incomplete understanding of the many compensatory molecular changes that occur when one treats with a therapy. In (1) we explored a panel of resistance mechanisms to RTK inhibitors, showed that coordinate JNK/Erk/Akt measurement was essential to predict cellular response, and showed that the resistance mechanism's effects could be explained through their effects on these pathways. In (3) we showed that a complication of targeting autocrine growth factor signaling is the length-scales on which ligand release and recapture occur. Through a diffusion reaction model, we instead predicted and showed that inhibiting ligand release through protease inhibition is much more effective. In (2), we showed that a common consequence of kinase inhibitors is reduced proteolytic shedding on the cell surface. This change switches the kinase dependence of cells, in turn driving resistance to therapy (in large part via AXL). These results highlight the complexity underlying targeted inhibitor response and demonstrate methods to understand and overcome it.

1. Manole, S., E.J. Richards, **A.S. Meyer**. JNK pathway activation modulates acquired resistance to EGFR/HER2 targeted therapies. *Cancer Research*. 2016 Sept 15; 76 (18): 5219-5228. PMCID: 5026573.
2. Miller, M.A., M.J. Oudin, R.J. Sullivan, S.J. Wang, **A.S. Meyer**, H. Im, D.T. Frederick, J. Tadros, L.G. Griffith, H. Lee, R. Weissleder, K.T. Flaherty, F.B. Gertler, D.A. Lauffenburger. (2016). Reduced Proteolytic Shedding of Receptor Tyrosine Kinases is a Post-Translational Mechanism of Kinase Inhibitor Resistance. *Cancer Discovery*, 6(4):331–333, April 2016. PMCID: 5087317.
3. M.A. Miller, M.L. Moss, G. Powell, R. Petrovich, L. Edwards, **A.S. Meyer**, Linda G. Griffith, D.A. Lauffenburger. Targeting autocrine HB-EGF signaling with specific ADAM12 inhibition using recombinant ADAM12 prodomain. *Scientific Reports*, 5:15150 EP –, October 2015. PMCID: 4609913.
4. Schwartz, A.D., L.E. Barney, L.E. Jansen, T.V. Nguyen, C.L. Hall, **A.S. Meyer**, S. Peyton. (2017). A Biomaterial Screening Approach to Reveal Microenvironmental Mechanisms of Drug Resistance. *Integrative Biology*. 2017 Dec 11;9(12):912-924. PMCID: PMC5725273.

## Migration and metastasis mechanisms

Invasion and dissemination of cells underlie many diseases including breast cancer. Studying these processes is challenging due to their complex regulation and the multiple biophysical steps involved. In earlier work, we quantified the growth factor responsiveness of cell migration overall and individual processes involved in cell migration, then compared them to 3D invasion through extracellular matrix (1). This identified that these same individual processes still regulated migration in 3D, but that the overall rate-limiting steps and thus migration response were different. By studying the signaling (3) and protease (2) regulation of migration, we then linked these processes to the invasive response to identify therapeutic approaches.

1. **Meyer, A.S.**, S.K. Hughes-Alford, J.E. Kay, A. Castillo, A. Wells, F.B. Gertler, D.A. Lauffenburger (2012). 2D protrusion but not motility predicts growth factor-induced cancer cell migration in 3D collagen. *Journal of Cell Biology*, 197(6), 721-729. PMCID: 3373410.
2. Miller, M.A.<sup>‡</sup>, **A.S. Meyer**<sup>‡</sup>, M. Beste, Z. Lasisi, S. Reddy, Jeng, K., Chen, C.-H., Han, J., Isaacson, K., Griffith, L.G., Lauffenburger, D.A. (2013). ADAM-10 and -17 regulate endometriotic cell migration via concerted ligand and receptor shedding feedback on kinase signaling. *Proc. Natl. Acad. Sci. USA*, 110(22), E2074-E2083. PMCID: 3670354.
3. Kim, H.D., **Meyer, A.S.**, Wagner, J.P., Alford, S.K., Wells, A., Gertler, F.B., Lauffenburger, D.A. (2011). Signaling network state predicts Twist-mediated effects on breast cell migration across diverse growth factor contexts. *Molecular & Cellular Proteomics*, 10(11), M111.008433. PMCID: 3226401.
4. Riquelme, D.N., **A.S. Meyer**, M. Barzik, A. Keating, F.B. Gertler. (2015). Selectivity in Subunit Composition of Ena/VASP Tetramers. *Bioscience Reports*, 2015. PMCID: 4721544.

<sup>‡</sup>Equal contribution.

**Complete List of Published Work in My Bibliography:** <http://1.usa.gov/1So8BFr>

## D. Research Support

### Ongoing Research Support

NIH U01-CA215709

9/01/2017 – 8/30/2022

Precision Lung Cancer Therapy Design Through Multiplexed Adapter Measurement

We propose to develop a global picture of bypass resistance mediated by receptor tyrosine kinases (RTKs) not targeted by therapy. By examining multiple RTKs variable in their resistance-promoting capacity at once, we will be able to determine features essential to the development of resistance. Examining receptor-proximal interactions, we will identify causally-important interactions that can be measured to identify which receptors are directing resistance.

Role: Co-PI

[REDACTED]

7/1/2017 – 6/30/2019

[REDACTED]

This project aims to use a computational model to direct design of new inhibitors for the TAM receptors. Using these well-characterized compounds, we will examine the *in vivo* effects of inhibiting different TAM receptor complements.

Role: PI

NIH DP5-OD019815

9/22/2014 – 9/1/2019

Adapter-Layer RTK Signaling: Basic Understanding & Targeted Drug Resistance

The goal of this project is to study sets of resistance mechanisms to RTK-targeted therapies, in order to identify commonalities and ways to determine which mechanism may be driving individual tumors.

Role: PI

## Completed Research Support

6/1/2015 – 6/1/2016

Quantitative and Multiplexed Tools for Probing G-Protein Coupled Receptor Activation

The goal of this project was to develop a novel multiplexed G-protein activation assay able to globally assess their activity.

Role: Co-PI

**BIOGRAPHICAL SKETCH**  
**DO NOT EXCEED FIVE PAGES.**

NAME: Nimmerjahn, Falk

eRA COMMONS USER NAME (credential, e.g., agency login): [REDACTED]

POSITION TITLE: Full Professor of Immunology and Chairman of the Institute of Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

| INSTITUTION AND LOCATION                                                   | DEGREE<br>(if applicable) | Completion<br>Date<br>MM/YYYY | FIELD OF STUDY           |
|----------------------------------------------------------------------------|---------------------------|-------------------------------|--------------------------|
| Friedrich-Alexander University Erlangen-Nuremberg, Germany                 | Diploma (Master)          | 05-1998                       | Immunology, genetics     |
| Ludwig-Maximilians University Munich                                       | PhD                       | 05-2002                       | Tumor immunology         |
| National Research Center for Environment and Health (GSF), Munich, Germany | scientist                 | 05-2004                       | Immunology, Virology     |
| The Rockefeller University, New York, USA                                  | Postdoc                   | 08-2007                       | Immunology, autoimmunity |

**A. Personal Statement**

I have a long standing research interest in the mechanisms underlying antibody activity. We were one of the first groups showing how IgG subclasses and IgG glycovariants not only modulate the pro- but also the anti-inflammatory activity of IgG (1, 2). Moreover, our work on how different mouse and human IgG subclasses are glycosylated and how they mediate their activity in glycosylation dependent and independent ways may be of major relevance for polyclonal and complex antibody responses induced by bacterial or viral infections (3, 4). Thus, I feel confident that my area of expertise will be of considerable value in this project.

1) Seeling, M., Bruckner, C., and Nimmerjahn, F. (2017). Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity? Nat Rev Rheumatol 13, 621-630.10.1038/nrrheum.2017.146

2) Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310, 1510-1512.

3) Kao, D., Danzer, H., Collin, M., Gross, A., Eichler, J., Stambuk, J., Lauc, G., Lux, A., and Nimmerjahn, F. (2015). A Monosaccharide Residue Is Sufficient to Maintain Mouse and Human IgG Subclass Activity and Directs IgG Effector Functions to Cellular Fc Receptors. Cell Rep 13, 2376-2385.10.1016/j.celrep.2015.11.0276)

4) Schwab, I., Lux, A., and Nimmerjahn, F. (2015). Pathways Responsible for Human Autoantibody and Therapeutic Intravenous IgG Activity in Humanized Mice. Cell Rep 13, 610-620.

**B. Positions and Honors****Positions and Employment**Positions

2007-2010: Associate professor of immunology, Medical Department 3, University of Erlangen-Nuremberg, Germany

Since 2010: Full professor and chairman, Institute of Genetics, Department of Biology, University of Erlangen-Nuremberg, Germany

Since 2017: Head of the Department of Biology at the University of Erlangen-Nuremberg, Germany

Fellowships

2005-2007 Fellow of the Cancer Research Institute, New York

|            |                                                           |
|------------|-----------------------------------------------------------|
| 2007-2012  | Fellow of the Bavarian Genome Research Network            |
| Since 2006 | Fellow of the German Society of Immunology                |
| Since 2009 | Fellow of the Henry G. Kunkel society, New York           |
| Since 2016 | Member of the American Association of Immunologists (AAI) |

### **Other Experience and Professional Memberships**

|      |                                                                                                                                             |
|------|---------------------------------------------------------------------------------------------------------------------------------------------|
| 2003 | Ph.D. award, Helmholtz Centre Munich and Ludwig-Maximilians University Munich for the best Ph.D. thesis in immunology and molecular biology |
| 2008 | Excellence in research award from the European Macrophage and Dendritic Cell society                                                        |
| 2008 | Pro-Scientia award of the Eckhart Buddecke foundation                                                                                       |
| 2009 | Paul Ehrlich and Ludwig Darmstädter award                                                                                                   |

## **C. Contribution to Science**

1. My group has a long standing interest in deciphering how cellular Fc-receptors contribute to the activity of immunoglobulin G (IgG) antibodies. Over the last 12 years we have published some of the key papers demonstrating that cytotoxic IgG antibodies mediate their activity mainly via cellular Fc-receptors and not via the activation of the complement pathway. These findings have been reproduced by many other groups and have led to the development of second generation therapeutic antibodies with an enhanced affinity to cellular Fc-receptors.

- a. Lehmann, B., Biburger, M., Bruckner, C., Ipsen-Escobedo, A., Gordan, S., Lehmann, C., Voehringer, D., Winkler, T., Schaft, N., Dudziak, D., Sirbu, H., Weber, G.F., and Nimmerjahn, F. (2017). Tumor location determines tissue-specific recruitment of tumor-associated macrophages and antibody-dependent immunotherapy response. *Sci Immunol* 2.10.1126/sciimmunol.aah6413
- b. Schwab, I., Lux, A., and Nimmerjahn, F. (2015). Pathways Responsible for Human Autoantibody and Therapeutic Intravenous IgG Activity in Humanized Mice. *Cell Rep* 13, 610-620.
- c. Nimmerjahn, F., and Ravetch, J.V. (2005). Divergent immunoglobulin G subclass activity through selective Fc receptor binding. *Science* 310, 1510-1512.
- d. Nimmerjahn, F., Bruhns, P., Horiuchi, K., and Ravetch, J.V. (2005). Fc gamma RIV: A novel FcR with distinct IgG subclass specificity. *Immunity* 23, 41-51.

2. Another key aspect of our research is to understand which effector cells are responsible for mouse and human IgG subclass activity. This work has led to a change in our current models of how target cells become depleted by cytotoxic antibodies. While the long standing assumption was that natural killer cells are the main cell type involved in antibody dependent cell mediated cytotoxicity (ADCC) it has become established that at least in classical and humanized mouse model systems not NK cells but rather cells of the mononuclear phagocytic system, including tissue resident macrophages and monocytes are key effector cells in this pathway. Within this framework, our studies have also resulted in a new understanding of how the pro-inflammatory activity of IgG is modulated and how glycosylation of IgG impacts its activity. We were able to show that IgG antibodies lacking fucose residues in their sugar domain have a more than ten-fold increased affinity for mouse FcRIV and human FcRIIIa, respectively. This increased affinity translated into enhanced FcR dependent effector functions and this concept has now been translated into the clinic in the form of second generation therapeutic antibodies with increased affinity for activating and reduced affinity for the inhibitory FcRIIb.

- a. Lehmann, C.H.K., Baranska, A., Heidkamp, G.F., Heger, L., Neubert, K., Luhr, J.J., Hoffmann, A., Reimer, K.C., Bruckner, C., Beck, S., Seeling, M., Kiessling, M., Soulat, D., Krug, A.B., Ravetch, J.V., Leusen, J.H.W., Nimmerjahn, F., and Dudziak, D. (2017). DC subset-specific induction of T cell responses upon antigen uptake via Fc gamma receptors in vivo. *J Exp Med* 214, 1509-1528.10.1084/jem.20160951; equal contribution senior author
- b. Lux, A., Seeling, M., Baerenwaldt, A., Lehmann, B., Schwab, I., Repp, R., Meidenbauer, N., Mackensen, A., Hartmann, A., Heidkamp, G., et al. (2014). A humanized mouse identifies the bone marrow as a niche with low therapeutic IgG activity. *Cell Rep* 7, 236-248.



- c. Kao, D., Danzer, H., Collin, M., Gross, A., Eichler, J., Stambuk, J., Lauc, G., Lux, A., and Nimmerjahn, F. (2015). A Monosaccharide Residue Is Sufficient to Maintain Mouse and Human IgG Subclass Activity and Directs IgG Effector Functions to Cellular Fc Receptors. *Cell Rep* 13, 2376-2385.
- d. Lux, A., Yu, X., Scanlan, C.N., and Nimmerjahn, F. (2013). Impact of immune complex size and glycosylation on IgG binding to human Fcγ receptors. *J Immunol* 190, 4315-4323.

3. A third focus of our work, which has resulted in a new understanding of how the pro-inflammatory activity of IgG is modulated, is to define how glycosylation of IgG impacts its activity. We were able to show that IgG antibodies lacking fucose residues in their sugar domain have a more than ten-fold increased affinity for mouse FcγRIV and human FcγRIIIa, respectively. This increased affinity translated into enhanced FcR dependent effector functions and this concept has now been translated into the clinic in the form of second generation therapeutic antibodies with increased affinity for activating and reduced affinity for the inhibitory FcγRIIb. Further along these lines we were able to show that lack of galactosylation does not impact the pro-inflammatory activity of IgG. In contrast the size of an immune complex is a major factor determining the pro-inflammatory activity of IgG subclasses and may even diminish the influence of differentially glycosylated IgG variants with respect to enhanced or decreased binding to individual activating FcRs.

- a. Kao, D., Danzer, H., Collin, M., Gross, A., Eichler, J., Stambuk, J., Lauc, G., Lux, A., and Nimmerjahn, F. (2015). A Monosaccharide Residue Is Sufficient to Maintain Mouse and Human IgG Subclass Activity and Directs IgG Effector Functions to Cellular Fc Receptors. *Cell Rep* 13, 2376-2385.
- b. Lux, A., Yu, X., Scanlan, C.N., and Nimmerjahn, F. (2013). Impact of immune complex size and glycosylation on IgG binding to human Fcγ receptors. *J Immunol* 190, 4315-4323.
- c. Karsten, C.M., Pandey, M.K., Figge, J., Kilchenstein, R., Taylor, P.R., Rosas, M., McDonald, J.U., Orr, S.J., Berger, M., Petzold, D., Blanchard, V., Winkler, A., Hess, C., Reid, D.M., Majoul, I.V., Strait, R.T., Harris, N.L., Kohl, G., Wex, E., Ludwig, R., Zillikens, D., Nimmerjahn, F., Finkelman, F.D., Brown, G.D., Ehlers, M., and Kohl, J. (2012). Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIb and dectin-1. *Nat Med* 18, 1401-1406.10.1038/nm.2862
- d. Nimmerjahn, F., Anthony, R.M., and Ravetch, J.V. (2007). Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. *Proceedings of the National Academy of Sciences of the United States of America* 104, 8433-8437.

## Complete list of Published Work in My Bibliography

<https://www.ncbi.nlm.nih.gov/pubmed/?term=nimmerjahn+f>

## D. Additional information Research Support and/or Scholastic Performance

All current grants were awarded by [REDACTED]

### Ongoing Research Support

1. Applicant: Falk Nimmerjahn  
 Sponsor: [REDACTED]  
 Project title: Understanding the role of the human inhibitory Fcγ-receptor for autoreactive and protective humoral immune responses *in vivo*  
 Funding code: [REDACTED]  
 Duration: 07/2017-06/2021
2. Applicant: Falk Nimmerjahn and Diana Dudziak  
 Sponsor: [REDACTED]  
 Project title: Investigating the molecular and cellular pathways of intravenous immunoglobulin G mediated resolution of established autoimmune inflammation  
 Funding code: [REDACTED]

Duration: 07/2015-06/2019

3. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Role of B cells for the initiation of systemic autoimmune diseases  
Funding code: [REDACTED]  
Duration: 07/2015-06/2019
4. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Human IgG Subclass Glycosylation  
Funding code: [REDACTED]  
Duration: 07/2018-06/2021
5. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: IgG glycosylation as a regulative factor controlling onset of RA  
Funding code: [REDACTED]  
Duration: 07/2019-06/2022

#### Completed Research Support:

1. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Investigating the mechanism of antibody mediated effector functions in vivo  
Funding code: [REDACTED]  
Duration: 2008-2016
2. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Influence of immunoglobulin G on osteoclast and osteoblast development, activation and effector functions  
Funding code: [REDACTED]  
Duration: 2010-2016
3. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Impact of deregulated inhibitory Fc-receptor function on the humoral immune system in humanized mice  
Funding code: [REDACTED]  
Duration: 2007-2013
4. Applicant: Falk Nimmerjahn  
Sponsor: [REDACTED]  
Project title: Establishing a humanized mouse model system to study human humoral immune responses in vivo  
Funding code: not applicable  
Duration: 2007-2012

## PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

### 1. Vertebrate Animals Section

Are vertebrate animals euthanized? ☒ Yes ☐ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

☒ Yes ☐ No

If "No" to AVMA guidelines, describe method and provide scientific justification

.....

### 2. \*Program Income Section

\*Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

| *Budget Period | *Anticipated Amount (\$) | *Source(s) |
|----------------|--------------------------|------------|
|----------------|--------------------------|------------|

## PHS 398 Cover Page Supplement

### 3. Human Embryonic Stem Cells Section

\*Does the proposed project involve human embryonic stem cells? ☐ Yes ☒ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: [http://grants.nih.gov/stem\\_cells/registry/current.htm](http://grants.nih.gov/stem_cells/registry/current.htm). Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

### 4. Inventions and Patents Section (Renewal applications)

\*Inventions and Patents: ☐ Yes ☐ No

If the answer is "Yes" then please answer the following:

\*Previously Reported: ☐ Yes ☐ No

### 5. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

\*First Name:

Middle Name:

\*Last Name:

Suffix:

☐ Change of Grantee Institution

\*Name of former institution:

## PHS 398 Modular Budget

OMB Number: 0925-0001  
Expiration Date: 03/31/2020

| Budget Period: 1                                                            |                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
|-----------------------------------------------------------------------------|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Start Date: 12/01/2019    End Date: 11/30/2020                              |                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
| A. Direct Costs                                                             |                                                   | <div style="text-align: right;">Funds Requested (\$)</div> <div style="text-align: right;">Direct Cost less Consortium Indirect (F&amp;A)* <span style="background-color: black; color: black;">[REDACTED]</span></div> <div style="text-align: right;">Consortium Indirect (F&amp;A) <span style="background-color: black; color: black;">[REDACTED]</span></div> <div style="text-align: right; border-top: 1px solid black;">Total Direct Costs* <span style="background-color: black; color: black;">[REDACTED]</span></div> |                                                                        |                                                                                                                                                                                                                |
| B. Indirect (F&A) Costs                                                     |                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
|                                                                             | Indirect (F&A) Type                               | Indirect (F&A) Rate (%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Indirect (F&A) Base (\$)                                               | Funds Requested (\$)                                                                                                                                                                                           |
| 1.                                                                          | Research On Campus                                | <span style="background-color: black; color: black;">[REDACTED]</span>                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <span style="background-color: black; color: black;">[REDACTED]</span> | <span style="background-color: black; color: black;">[REDACTED]</span>                                                                                                                                         |
| 2.                                                                          | <div style="border-top: 1px dotted black;"></div> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
| 3.                                                                          | <div style="border-top: 1px dotted black;"></div> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
| 4.                                                                          | <div style="border-top: 1px dotted black;"></div> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        |                                                                                                                                                                                                                |
| Cognizant Agency<br><small>(Agency Name, POC Name and Phone Number)</small> |                                                   | DHHS, Janet Turner, <span style="background-color: black; color: black;">[REDACTED]</span>                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                        |                                                                                                                                                                                                                |
| Indirect (F&A) Rate Agreement Date                                          |                                                   | 05/03/2017                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                        | <div style="text-align: right;">Total Indirect (F&amp;A) Costs</div> <div style="text-align: right; border-top: 1px solid black;"><span style="background-color: black; color: black;">[REDACTED]</span></div> |
| C. Total Direct and Indirect (F&A) Costs (A + B)                            |                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                        | <div style="text-align: right;">Funds Requested (\$)</div> <div style="text-align: right; border-top: 1px solid black;"><span style="background-color: black; color: black;">[REDACTED]</span></div>           |

## PHS 398 Modular Budget

| Budget Period: 2                                                            |                         |                                                                                                 |                                                                |
|-----------------------------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Start Date: 12/01/2020    End Date: 11/30/2021                              |                         |                                                                                                 |                                                                |
| A. Direct Costs                                                             |                         | Direct Cost less Consortium Indirect (F&A)*<br>Consortium Indirect (F&A)<br>Total Direct Costs* | Funds Requested (\$)<br>[REDACTED]<br>[REDACTED]<br>[REDACTED] |
| B. Indirect (F&A) Costs                                                     |                         |                                                                                                 |                                                                |
| Indirect (F&A) Type                                                         | Indirect (F&A) Rate (%) | Indirect (F&A) Base (\$)                                                                        | Funds Requested (\$)                                           |
| 1. Research On Campus                                                       | [REDACTED]              | [REDACTED]                                                                                      | [REDACTED]                                                     |
| 2. ....                                                                     |                         |                                                                                                 |                                                                |
| 3. ....                                                                     |                         |                                                                                                 |                                                                |
| 4. ....                                                                     |                         |                                                                                                 |                                                                |
| Cognizant Agency<br><small>(Agency Name, POC Name and Phone Number)</small> |                         | DHHS, Janet Turner, [REDACTED]                                                                  |                                                                |
| Indirect (F&A) Rate Agreement Date                                          | 05/03/2017              | Total Indirect (F&A) Costs                                                                      | [REDACTED]                                                     |
| C. Total Direct and Indirect (F&A) Costs (A + B)                            |                         | Funds Requested (\$) [REDACTED]                                                                 |                                                                |

## PHS 398 Modular Budget

| Budget Period: 3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|---------------------|-------------------------|--------------------------|----------------------|-----------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|---------|--|--|--|---------|--|--|--|---------|--|--|--|
| Start Date: 12/01/2021    End Date: 11/30/2022                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p><b>A. Direct Costs</b></p> <div style="margin-top: 20px;"> <div style="display: flex; justify-content: space-between;"> <div style="width: 80%;"> <p style="margin: 0;">Direct Cost less Consortium Indirect (F&amp;A)*</p> <p style="margin: 0;">Consortium Indirect (F&amp;A)</p> <p style="margin: 0; border-top: 1px solid black;">Total Direct Costs*</p> </div> <div style="width: 20%; text-align: right;"> <p style="margin: 0;">Funds Requested (\$)</p> <div style="background-color: black; width: 100px; height: 20px; margin: 0;"></div> <div style="background-color: black; width: 100px; height: 20px; margin: 0;"></div> <div style="background-color: black; width: 100px; height: 20px; margin: 0;"></div> </div> </div> </div> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| <p><b>B. Indirect (F&amp;A) Costs</b></p> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 40%; text-align: left;">Indirect (F&amp;A) Type</th> <th style="width: 15%; text-align: center;">Indirect (F&amp;A) Rate (%)</th> <th style="width: 20%; text-align: center;">Indirect (F&amp;A) Base (\$)</th> <th style="width: 25%; text-align: right;">Funds Requested (\$)</th> </tr> </thead> <tbody> <tr> <td>1. Research On Campus</td> <td style="text-align: center;"><div style="background-color: black; width: 30px; height: 20px;"></div></td> <td style="text-align: center;"><div style="background-color: black; width: 50px; height: 20px;"></div></td> <td style="text-align: right;"><div style="background-color: black; width: 50px; height: 20px;"></div></td> </tr> <tr> <td>2. ....</td> <td></td> <td></td> <td></td> </tr> <tr> <td>3. ....</td> <td></td> <td></td> <td></td> </tr> <tr> <td>4. ....</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <div style="margin-top: 20px;"> <p>Cognizant Agency <span style="float: right;">DHHS, Janet Turner, <div style="background-color: black; width: 70px; height: 20px;"></div></span></p> <p style="font-size: small;">(Agency Name, POC Name and Phone Number)</p> </div> <div style="margin-top: 20px; display: flex; justify-content: space-between;"> <div>Indirect (F&amp;A) Rate Agreement Date    05/03/2017</div> <div style="text-align: right;"> <p>Total Indirect (F&amp;A) Costs</p> <div style="background-color: black; width: 50px; height: 20px;"></div> </div> </div> |                                                                         |                                                                         |                                                                         | Indirect (F&A) Type | Indirect (F&A) Rate (%) | Indirect (F&A) Base (\$) | Funds Requested (\$) | 1. Research On Campus | <div style="background-color: black; width: 30px; height: 20px;"></div> | <div style="background-color: black; width: 50px; height: 20px;"></div> | <div style="background-color: black; width: 50px; height: 20px;"></div> | 2. .... |  |  |  | 3. .... |  |  |  | 4. .... |  |  |  |
| Indirect (F&A) Type                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Indirect (F&A) Rate (%)                                                 | Indirect (F&A) Base (\$)                                                | Funds Requested (\$)                                                    |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| 1. Research On Campus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <div style="background-color: black; width: 30px; height: 20px;"></div> | <div style="background-color: black; width: 50px; height: 20px;"></div> | <div style="background-color: black; width: 50px; height: 20px;"></div> |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| 2. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| 3. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| 4. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |
| <div style="display: flex; justify-content: space-between; margin-top: 20px;"> <div style="width: 60%;"> <p><b>C. Total Direct and Indirect (F&amp;A) Costs (A + B)</b></p> </div> <div style="width: 40%; text-align: right;"> <p>Funds Requested (\$)</p> <div style="background-color: black; width: 100px; height: 20px;"></div> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                         |                                                                         |                                                                         |                     |                         |                          |                      |                       |                                                                         |                                                                         |                                                                         |         |  |  |  |         |  |  |  |         |  |  |  |

## PHS 398 Modular Budget

| Budget Period: 4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                          |                                                                          |                                                                          |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------------|-------------------------|--------------------------|----------------------|-----------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|---------|--|--|-------|---------|--|--|-------|---------|--|--|-------|
| Start Date: 12/01/2022    End Date: 11/30/2023                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                          |                                                                          |                                                                          |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> <p><b>A. Direct Costs</b></p> </div> <div style="width: 60%; text-align: right;"> <p>Funds Requested (\$)</p> <div style="display: flex; justify-content: flex-end; align-items: flex-end;"> <div style="text-align: right; margin-right: 20px;"> Direct Cost less Consortium Indirect (F&amp;A)*<br/> Consortium Indirect (F&amp;A)<br/> Total Direct Costs* </div> <div style="border-top: 1px solid black; width: 100px; text-align: right;"> <div style="background-color: black; width: 100px; height: 1.2em; margin-bottom: 2px;"></div> <div style="background-color: black; width: 100px; height: 1.2em; margin-bottom: 2px;"></div> <div style="background-color: black; width: 100px; height: 1.2em;"></div> </div> </div> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                          |                                                                          |                                                                          |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
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| Indirect (F&A) Type                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Indirect (F&A) Rate (%)                                                  | Indirect (F&A) Base (\$)                                                 | Funds Requested (\$)                                                     |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| 1. Research On Campus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <div style="background-color: black; width: 30px; height: 1.2em;"></div> | <div style="background-color: black; width: 50px; height: 1.2em;"></div> | <div style="background-color: black; width: 50px; height: 1.2em;"></div> |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| 2. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                          |                                                                          | .....                                                                    |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| 3. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                          |                                                                          | .....                                                                    |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| 4. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                          |                                                                          | .....                                                                    |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p><b>C. Total Direct and Indirect (F&amp;A) Costs (A + B)</b></p> </div> <div style="width: 40%; text-align: right;"> <p>Funds Requested (\$)</p> <div style="border-top: 1px solid black; width: 100px; text-align: right;"> <div style="background-color: black; width: 100px; height: 1.2em;"></div> </div> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                          |                                                                          |                                                                          |                     |                         |                          |                      |                       |                                                                          |                                                                          |                                                                          |         |  |  |       |         |  |  |       |         |  |  |       |



## PHS 398 Modular Budget

| Budget Period: 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------|----------------------|---------------------------------------------|-------------------------|---------------------------|----------------------|----------------------------|------------|------------|------------|---------|--|--|--|---------|--|--|--|---------|--|--|--|
| Start Date: 12/01/2023    End Date: 11/30/2024                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> <p><b>A. Direct Costs</b></p> </div> <div style="width: 60%; text-align: right;"> <p>Funds Requested (\$)</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">Direct Cost less Consortium Indirect (F&amp;A)*</td> <td style="width: 20%; text-align: right;">[REDACTED]</td> </tr> <tr> <td>Consortium Indirect (F&amp;A)</td> <td style="text-align: right;">[REDACTED]</td> </tr> <tr> <td><b>Total Direct Costs*</b></td> <td style="text-align: right; border-top: 1px solid black;">[REDACTED]</td> </tr> </table> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                         |                          |                      | Direct Cost less Consortium Indirect (F&A)* | [REDACTED]              | Consortium Indirect (F&A) | [REDACTED]           | <b>Total Direct Costs*</b> | [REDACTED] |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| Direct Cost less Consortium Indirect (F&A)*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | [REDACTED]              |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| Consortium Indirect (F&A)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | [REDACTED]              |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| <b>Total Direct Costs*</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | [REDACTED]              |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| <p><b>B. Indirect (F&amp;A) Costs</b></p> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 40%;">Indirect (F&amp;A) Type</th> <th style="width: 15%;">Indirect (F&amp;A) Rate (%)</th> <th style="width: 20%;">Indirect (F&amp;A) Base (\$)</th> <th style="width: 25%;">Funds Requested (\$)</th> </tr> </thead> <tbody> <tr> <td>1. Research On Campus</td> <td style="text-align: center;">[REDACTED]</td> <td style="text-align: center;">[REDACTED]</td> <td style="text-align: center;">[REDACTED]</td> </tr> <tr> <td>2. ....</td> <td></td> <td></td> <td></td> </tr> <tr> <td>3. ....</td> <td></td> <td></td> <td></td> </tr> <tr> <td>4. ....</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <div style="margin-top: 10px;"> <p>Cognizant Agency                      DHHS, Janet Turner, [REDACTED]</p> <p><small>(Agency Name, POC Name and Phone Number)</small></p> </div> <div style="margin-top: 10px; display: flex; justify-content: space-between;"> <div>Indirect (F&amp;A) Rate Agreement Date      05/03/2017</div> <div style="text-align: right;"> <p><b>Total Indirect (F&amp;A) Costs</b></p> <p style="border-top: 1px solid black; display: inline-block; width: 100px;">[REDACTED]</p> </div> </div> |                         |                          |                      | Indirect (F&A) Type                         | Indirect (F&A) Rate (%) | Indirect (F&A) Base (\$)  | Funds Requested (\$) | 1. Research On Campus      | [REDACTED] | [REDACTED] | [REDACTED] | 2. .... |  |  |  | 3. .... |  |  |  | 4. .... |  |  |  |
| Indirect (F&A) Type                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Indirect (F&A) Rate (%) | Indirect (F&A) Base (\$) | Funds Requested (\$) |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| 1. Research On Campus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | [REDACTED]              | [REDACTED]               | [REDACTED]           |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| 2. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| 3. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| 4. ....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;"> <p><b>C. Total Direct and Indirect (F&amp;A) Costs (A + B)</b></p> </div> <div style="width: 40%; text-align: right;"> <p>Funds Requested (\$)</p> <p style="border-top: 1px solid black; display: inline-block; width: 100px;">[REDACTED]</p> </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                         |                          |                      |                                             |                         |                           |                      |                            |            |            |            |         |  |  |  |         |  |  |  |         |  |  |  |

## PHS 398 Modular Budget

| Cumulative Budget Information                                                              |                                                  |
|--------------------------------------------------------------------------------------------|--------------------------------------------------|
| <b>1. Total Costs, Entire Project Period</b>                                               |                                                  |
| Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period (\$) | [REDACTED]                                       |
| Section A, Total Consortium Indirect (F&A) for Entire Project Period (\$)                  | [REDACTED]                                       |
| Section A, Total Direct Costs for Entire Project Period (\$)                               | [REDACTED]                                       |
| Section B, Total Indirect (F&A) Costs for Entire Project Period (\$)                       | [REDACTED]                                       |
| Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period (\$)      | [REDACTED]                                       |
| <b>2. Budget Justifications</b>                                                            |                                                  |
| Personnel Justification                                                                    | personnel_justification1054050153.pdf            |
| Consortium Justification                                                                   | CONSORTIUM_JUSTIFICATION_V21054050154.pdf        |
| Additional Narrative Justification                                                         | Additional_Narrative_Justification1054050155.pdf |

## Personnel Justification

The budget request covers only expenses explicitly related to these projects. There is no overlap with other sources of support.

### Senior / Key Personnel

**Co-Principal Investigator—Aaron Meyer (17% effort or 1.5 summer months)** Dr. Meyer is responsible for all aspects of the project. He will directly supervise the work of the trainees within his laboratory, and be responsible for their training and mentorship. He will also be directly involved in generation and analysis of all data, and lead the computational analysis throughout. He will work closely with Dr. Nimmerjahn to ensure integration of the computational analysis and experimental validation. 30% of his effort will be allocated to the project and partial salary support is requested.

### Other Personnel

**Graduate Student Researcher—TBD (47.29% effort, 12 calendar months)** This graduate student will be centrally responsible for executing the computational analysis throughout the project, and especially Aims 1 and 3, alongside Dr. Meyer. This student will have prior computational experience, and will further develop his/her skill set. Commensurate salary support is requested, along with three quarters of tuition support per year.

**Graduate Student Researcher—TBD (47.29% effort, 7 calendar months)** This graduate student will focus on the human-murine homology model, and the factorization methods, contained within Aim 2. He/she will work alongside Dr. Meyer to execute this analysis and plan the validation experiments for these results. The student will have prior computational experience and will further develop his/her skill set. Commensurate salary support is requested, along with two quarters tuition support per year.

## CONSORTIUM JUSTIFICATION

This proposal includes a subcontract to Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), in Germany. FAU and the Nimmerjahn lab specifically are included in this proposal because they bring unique and vital, internationally-recognized expertise in the immunology of effector cell responses to IgG antibodies. Prof. Nimmerjahn's expertise extends to the experimental methods and reagents used throughout both Aim 1 and 3, the *in vivo* models used to validate the computational predictions within the project, and input regarding integration of the computational and experimental efforts. His participation is essential to effective execution of this project and will help to ensure the success of this proposal's important goal understanding how antibodies operate to direct target cell killing when present in combination. By accomplishing this goal, including FAU furthers the goals of the NIH more effectively than domestic alternatives.

### Total Costs

The total costs of this subaward are roughly [REDACTED] per year, or [REDACTED] over the five years of the award.

### Personnel

Falk Nimmerjahn, co-PI (1.20 calendar months) – Prof. Nimmerjahn will oversee the project, instruct the Post Doc, and participate in planning experimental strategies, data evaluation and writing of publications.

[REDACTED] (6 calendar months) – [REDACTED] will be responsible for planning and performing the experiments as described in the experimental plan, data interpretation and participate in writing of publications. As a postdoctoral trainee in my laboratory [REDACTED] will participate in career development programs at FAU very much alike FASEB's individual development plans. This includes mentoring by two faculty members, annual meetings to discuss career options and a variety of soft skill courses.

## **Additional Narrative Justification**

### **Graduate Student Remission**

The Graduate Student Researcher fee remission for residents for one academic quarter is [REDACTED]. We request funds for GSR fee remission for one graduate student for three academic quarters for all 4 years of this project. We anticipate tuition to increase at a 3% annual rate due to inflation.

### **Indirect Costs**

The indirect cost rate pertaining to this budget is [REDACTED] of the modified total direct costs (MTDC). For this proposal MTDC consists of total direct costs minus graduate student non-resident tuition and fee remissions for two graduate students.

## PHS 398 Research Plan

OMB Number: 0925-0001

Expiration Date: 03/31/2020

|                                                                                |                                         |
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| 2. Specific Aims                                                               | specific_aims_master_01221053682476.pdf |
| 3. Research Strategy*                                                          | strategy1053974460.pdf                  |
| 4. Progress Report Publication List                                            |                                         |
| Other Research Plan Section                                                    |                                         |
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| 10. Resource Sharing Plan(s)                                                   | resource_sharing_plan1053974453.pdf     |
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| Appendix                                                                       |                                         |
| 12. Appendix                                                                   |                                         |

## Specific Aims

Antibodies are crucial regulators of the immune response and particularly versatile therapeutic agents due to their ability to both bind 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 immune effector cell response. Those of the IgG isotype interact with FcγRs on effector cells. IgGs elicit effector response through multiple cell types (e.g., macrophages, monocytes) and through multiple processes, including removal of diseased cells through antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cell-mediated phagocytosis (ADCP). Many possible design parameters—constant region composition, FcγRs, cell populations, and antigen binding properties—make precisely predicting and manipulating effector function an elusive goal.

In a recently published study, we built a model of multivalent immune complex (IC—IgG-antigen complex) binding to FcγRs and showed that it can capture and predict experimentally measured binding and effector response of different IC compositions<sup>1</sup>. This model also predicted anti-tumor effector response to a single antibody of defined constant region *in vivo*. *Importantly, endogenous IgG responses are known to rely on antibodies of distinct constant region composition in combination. We hypothesize 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.* To identify potentially synergistic combinations of Fc domains, we propose to extend our model to incorporate binding and effector response of ICs comprised of IgG combinations.

**Aim 1: Validate a multivalent binding model's ability to predict FcγR binding to mixed IgG composition immune complexes.** *Hypothesis: An equilibrium multivalent binding model accurately represents FcγR interaction with ICs of mixed IgG composition.*

- Measure mixed composition synthetic IC binding to cells of defined FcγR expression.
- Generalize a multivalent binding model to account for ICs of mixed composition.
- Fit and verify that the model can predict effector response *in vitro*.

**Aim 2: Map human and murine IgG isotypes to one another according to conserved effector response.** *Hypothesis: Murine/human effector response regulation is conserved, even when single IgG isotypes are not.*

- Map IC composition to effector responses by tensor decomposition.
- Correlate murine and human effector responses according to similar cell population response.
- Verify this mapping predicts known, similar cross-species effector cell responses.

**Aim 3: Link IgG effects and *in vivo* efficacy to identify and verify synergistic IgG-elicited cell killing.** *Hypothesis: A binding model can identify synergistic effector interactions in vivo.*

- Regress single IgG treatments and *in vivo* cell clearance to identify synergistic combinations.
- Verify predicted cases of synergistic effector response *in vivo* within models of antibody-dependent, effector-mediated platelet and B cell depletion.
- Identify the relevant cell populations and mechanisms of synergistic effector response.

This investigation will considerably improve our ability to both engineer IgG with optimal effector cell killing response and inform how existing therapeutic and endogenous IgGs function. In particular, even existing monoclonal antibodies are mixtures of Fc compositions due to the cocktail of glycosylation forms present. A comprehensive view of how IgG Fc interact would therefore (1) improve our ability to match effector cell-mediated killing during antibody manufacturing, (2) provide insight into the role of the complex Fc cocktails created during an endogenous immune response, and (3) create the possibility of engineering logic into effector cell responses through antibody combinations.

## Significance

The therapeutic potential of antibodies is demonstrated by their status as a broad class of effective agents across autoimmune diseases, infection, and cancer. Their versatility is enabled through an antibody's selectivity toward target antigen as determined by its variable region, along with the ability to elicit effector cell responses depending upon the composition of its constant Fc region. Antibodies of the IgG type direct effector response by binding to the FcγR family of receptors. FcγR activation is driven by multiple IgG clustering the receptors. Depending upon the configuration of receptors, this interaction may promote or prevent effector response. Thus, the mechanism of FcγR activation ensures that multiple IgG are present whenever eliciting effector response.

One capability elicited by effector cells is clearance of infected or otherwise pathogenic cells. Clearance can occur through two functionally distinct mechanisms: antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cell-mediated phagocytosis (ADCP). However, both mechanisms are (1) regulated by the family of FcγRs present on effector cells, (2) modulated by the identity of the Fc region present on an IgG<sup>2</sup>, (3) performed by multiple cell types<sup>3,4</sup>, and (4) influenced by properties of antigen engagement<sup>5,6</sup>. This multilayered complexity is a central challenge to engineering antibodies with desirable cell-killing functions, as well as understanding successful and dysregulated endogenous immunity. Our team recently demonstrated that a multivalent binding model of immune complex (IC–IgG–antigen complex) binding to FcγRs accurately captured and could predict *in vitro* binding across various IgG isotypes<sup>1</sup>. Further, it could accurately predict antibody-elicited tumor cell killing *in vivo* across antibodies of varied isotype, glycosylation status, and FcγR knockout animals<sup>1</sup>. Directly quantifying and predicting cell clearance made it possible to accurately predict and optimize for antibody-mediated cell clearance regardless of whether it occurred by ADCC or ADCP.

Endogenous antibody responses universally involve Fc of diverse isotype and glycosylation in combination. *The central hypothesis of our proposal is that antibodies of different Fc composition, but identical antigen binding, can have properties other than the additive combination of either alone.* A consequence of this is that, within a mixture, minor species (e.g., glycosylation variant) can have an outsized effect promoting or preventing cell killing. Even when recombinantly manufacturing a single monoclonal therapeutic agent, heterogeneity exists in the glycosylation forms derived<sup>7,8</sup>. Knowledge of how these different forms influence the behavior of one another would allow one to increase or reduce cell killing by adjusting the mixture of glycosylation forms. This would also help guide evaluation of biosimilars by determining whether glycosylation forms present at small fractions might influence overall therapeutic efficacy. On the side of the effector cells involved in mediating therapeutic antibody dependent effects, it has become clear that in addition to NK cells (expressing only one activating FcγR (FcγRIIIA), tissue resident macrophages and bone marrow derived monocytes participate in cytotoxic antibody dependent target cell clearance. In contrast to NK cells, these myeloid cell subsets express all activating (excepting inflammatory monocytes lacking human (h)FcγRIIIA or mouse (m)FcγRIV) and the inhibitory FcγRIIB. Thus, mixed IC may trigger all or specific subsets of activating/inhibitory FcRs, resulting in a further complexity. Despite the ability of multiple activating FcRs on myeloid effector cells our previous studies have demonstrated that individual IgG subclasses, such as mIgG2a/2c for example, may mediate their activity through select activating FcγRs despite their capacity to bind to other activating FcRs<sup>2</sup>.

This work will only become more critical with recent advancements in our ability to experimentally characterize polyclonal IgG mixtures in ever finer resolution by making sense of this veritable data deluge<sup>9,10</sup>. Mapping interactions in effector response between pairs of antibodies will provide an essential first step toward more complex mixtures of Fc domains, and then integrating this information with variation in antigen binding. Thus, there is great potential to integrate this undertaking with other IgG engineering and antibody/effector cell characterization efforts.

## Innovation

This research is a convergence of immunology, data analysis, biophysics, simulation, and experiment to develop a more predictive, mechanism-based, and quantitative picture of IgG-mediated cell killing. Each of these areas is absolutely critical in combination to ensure the success of this proposal. In response to an antigen, our body creates a cocktail of antibodies of diverse class, glycosylation, and



antigen binding<sup>9,10</sup>. The biophysical properties of these molecules have been extensively characterized but almost always on a component-by-component basis<sup>11</sup>. The subsequent combinatorial complexity that arises ensures that the whole is more than the sum of its parts.

**Modeling innovation to calculate and visualize mixed IgG-FcγR binding** Innovative modeling and analytical methods herein address key challenges in computationally predicting and visualizing IgG-FcγR binding. Branching processes provide an elegant analytical approach for overcoming high degrees of combinatorial complexity to calculate overall binding state. These have been applied successfully to study aggregation phenomena such as antibody-antigen binding and polymer networks where there are analogous calculation challenges<sup>12,13</sup>. A second key challenge will be interpreting the high-dimensional space of possible IgG treatment combinations and response across cell populations. Tensor factorization provides an efficient and parsimonious representation of high-dimensional space and indeed has accepted use within the machine learning community for other problems of capturing high-dimensional relationships such as topic modeling<sup>14,15</sup>.

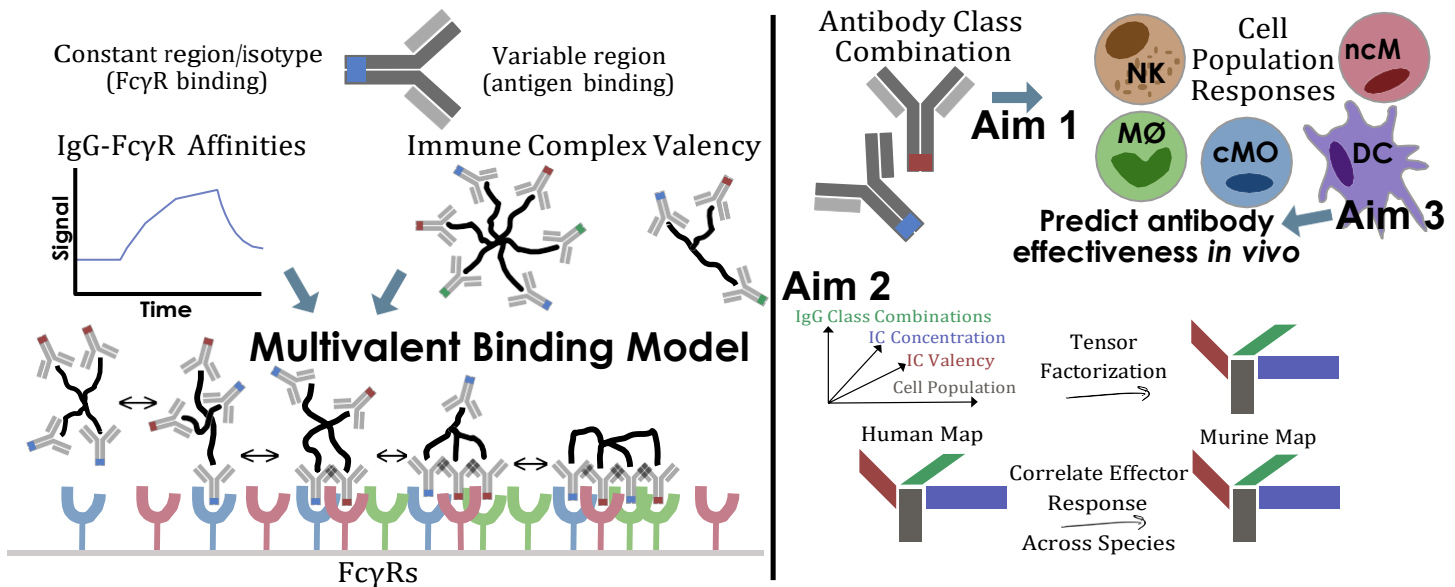
**Builds upon earlier theory on Fc receptor activation** Theoretical models have helped to understand Fc receptor activation, but critical gaps still exist in their application, especially when designing IgG therapies. Multivalent ligand/monovalent receptor binding models successfully represent activation of receptors such as FcεRI with similar binding configurations<sup>16-21</sup>. However, most cells express members of the FcγR family simultaneously in combination, meaning any manipulation of IC composition will necessarily have multivariate effects. Thus, while the underlying multivalent binding theory is long-standing, FcγR-IgG interactions are especially suited for developments in inference approaches to rigorously link these models to experimental observations and to visualize high-dimensional data<sup>22</sup>. Multivalent binding theory will be a critical companion to experiments mapping effector function; the baffling number of potential combinations preclude purely experimental searches or intuition from revealing precise answers<sup>9</sup>. For example, even considering 30 glycosylation variants, 4 IgG isotypes, pairs of two IgGs at 4 concentrations, and 5 antigen targets of varying valency, one is left with 9,600 design possibilities<sup>9,10</sup>.

The model used here is in essence a minimal pharmacologic model of IgG effector cell-elicited responses. By leaving out all but the most essential components, elegant pharmacologic models (e.g., competitive inhibition, additive interaction, etc) form the basis of analyzing compound effects from the most initial development stages through clinical evaluation<sup>23,24</sup>. Foundational models of effector-elicited responses will similarly allow for IgG therapies to be more rigorously engineered and evaluated.

**IgG-mediated logic** Identifying antibody constant regions with synergistic or antagonistic cell killing holds promise for more than just enhanced overall effector response. For example, a highly synergistic combination essentially provides AND logic between target antigens. Two constant regions that only lead to effector function in combination could help target cells for which a reliably specific antigen does not exist. In other words, if tumor cells are only unique in their expression of protein A and B, an anti-A/anti-B antibody combination would only signal for effector cell-elicited killing when both antigens are found in combination. This capability is similarly being pursued with chimeric antigen receptor cellular therapies in cancer, due to lack of completely specific tumor antigens, particularly in solid tumors<sup>25,26</sup>. By comparison, IgG-mediated logic would have significant benefits in cost, reliability, and likely toxicity as compared to cellular therapies. Other forms of logic may also be helpful and revealed by the approach here, such as A but not B to protect bystander antigen-expressing cells from an existing treatment. Therefore, synergistic constant region combinations hold promise both for enhancing the potency and avoiding side effects of therapeutic antibodies.

**Methods development relevant to other receptor-ligand families** Finally, the innovative methods here have immediate application in other areas of therapeutic engineering. Other immunotherapy and targeted therapy targets, such as the common γ-chain cytokines, FGF receptor tyrosine kinases, VEGF receptor tyrosine kinases, and bone morphogenic proteins, involve many ligands, large receptor families, and are expressed across many cell types<sup>27-31</sup>. The approach developed here—an activation model, parameterized through inference, and then mapped through tensor factorization—has immediate application in understanding the function of these other receptor families, learning how they respond to combinations of cues in the extracellular environment, and targeting their dysregulation.

## Approach



**Figure 1: Overview** (also see Gantt chart at end of proposal). Experimental IgG-FcγR binding measurements will be fit to a multivalent binding model extended to incorporate immune complexes with IgGs of mixed isotype and glycosylation. Binding and effector response predictions for these mixed isotype immune complexes will be tested *in vitro*. Using the computational model, we will then identify IgG combinations predicted to have synergistic effector-mediated killing and test on cell populations *in vitro* and in two *in vivo* models of platelet and B cell depletion. Using the model, we will also build a map of murine-human homology according to effector response.

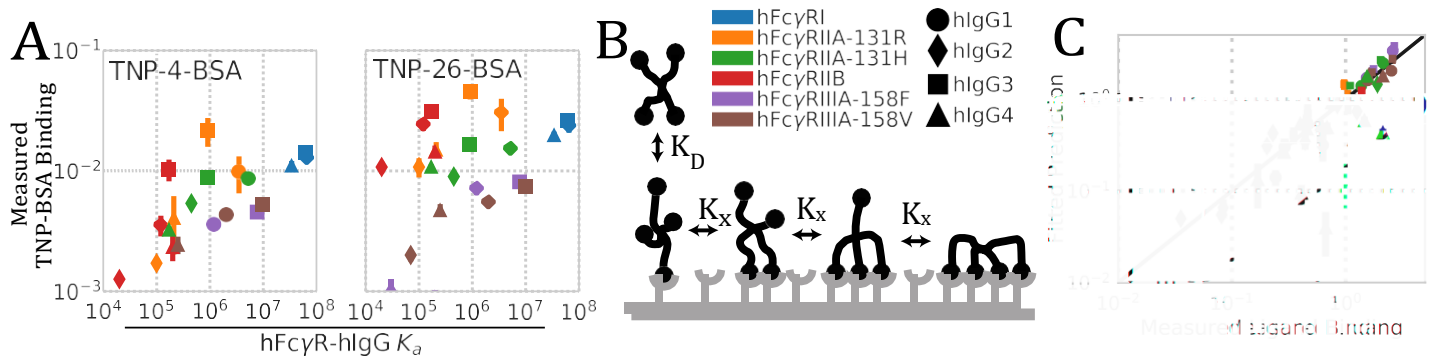
### Aim 1: Validate a multivalent binding model's ability to predict FcγR binding to mixed IgG composition immune complexes

**Rationale** FcγR activation and effector response occurs through multivalent immune complex (IC) binding and consequent receptor clustering. This aim will ensure that we are able to accurately model the binding and *in vitro* FcγR-dependent effector response of a cell population with defined FcγR expression, given we know the composition of an IC. At the same time, it will provide a helpful data compendium for examining the different factors that influence IC binding.

**1.1. Measure mixed composition synthetic IC binding to cells of defined FcγR expression** To start, we will utilize a panel of previously-generated CHO cell lines that express each human FcγRIA, FcγRIIA-131H, FcγRIIA-131R, FcγRIIA-158V, FcγRIIA-158F, or FcγRIIB individually (fig. 2)<sup>1,32</sup>. To ensure quantitative binding measurements, we will quantify receptor abundance in each cell line. These measurements are performed using by staining with FITC-coupled Abs directed against FcγRIA (CD64; clone 10.1; BD Pharmingen), FcγRIIA/IIB (CD32; clone 3D3; BD Pharmingen), and FcγRIIA (CD16; clone 3G8; BD Pharmingen). Absolute quantitation is obtained by comparison to a panel of beads with defined numbers of antibody binding sites in each experiment. Cell lines with multi-modal distributions of receptor expression or variance greater than 50% of the expression level will be sorted again for more precise expression.

We will assemble ICs using TNP(2,4,6-trinitrophenyl)-conjugated BSA at valencies of 4 and 26. Anti-TNP antibodies of each IgG isotype will be bound to the TNP-BSA. Instead of creating IgG-TNP-BSA complexes of one isotype as we performed previously<sup>1,32</sup>, we will use all pairs of 5:1 and 1:1 hIgG isotype mixtures, in duplicate. Binding will be quantified on cells using a PE-conjugated goat anti-human IgG F(ab')<sub>2</sub>. With four IgGs, six hFcγRs, and replicates, this corresponds to 528 independent binding measurements with which to ensure our model captures multi-IgG binding.

We anticipate that this data will show striking variation in the amount of cell binding, depending upon the valency of IC, affinity of each IgG used, ratio of IgGs present, and receptor expressed by the cell. In contrast to our earlier work, in this case there are two interaction affinities present, of an IgG present at higher and lower abundance. We expect this data will reveal that both affinities influence



**Figure 2: A two-step model captures IgG immune complex binding.** A) Measurements of IC binding to cells expressing a single FcγR. Error bars indicate standard error of triplicate measurements. B) Diagram of binding model. An initial monovalent interaction then leads to multivalent interaction with partition coefficient  $K_x$ . C) Fit versus actual binding measurement across all IgG/FcγR pairs. Note that the most divergent pair—hFcγRIIA-158F/hlgG4—was determined to be based on an unreliable affinity measurement (the equilibrium and kinetic binding assays in the source reference do not match for this case)<sup>33</sup>.

binding, which we can inspect by plotting matched cases wherein one IgG identity is held constant and the other varies. Additionally, we expect that the relative abundance of each IgG will matter, which we can inspect by plotting matched pairs where the only difference is the ratio between each IgG.

In addition to the isotype itself, the sugar moiety attached to the N297 residue in each individual IgG heavy chain can alter FcγR binding. Although several hundred IgG glycovariants may exist, the most striking effect in altered binding of an IgG glycovariant to FcγRs has been observed for fucosylated/afucosylated IgG variants, which bind with altered affinity to hFcγRIIA<sup>2,32,34</sup>. These IgG glycovariants can be generated via antibody production in cell lines deficient in the fucosyltransferase gene (LEC 13 CHO cells). To study how the abundance of certain IgG glycoforms affects FcγR binding, we will use pairs of 10:1, 3:1, and 1:1 hlgG1 glycovariant mixtures and study binding to hFcγRIIA-expressing cell lines like above in triplicate. Depending on the outcome of these experiments we plan to extend these studies to other human IgG isotypes. Of special interest are hlgG2 and hlgG4, which are considered low FcγR binders, yet it is unclear how the presence of different amounts of afucosylated glycovariants affects their functional activity. This set of data will be instrumental in assessing how the abundance of individual glycoforms in a mixture affects FcγR binding and effector functions (e.g., how much this can be allowed to change during manufacturing).

**1.2. Generalize a multivalent binding model to account for ICs of mixed composition** To model these binding data, we will extend our published model of IgG-FcγR engagement to account for IgG isotype and glycovariant mixtures<sup>1</sup>. Briefly, we model FcγR engagement as a two-step process, wherein an immune complex first binds to a single receptor with kinetics equal to those of monovalent binding (fig. 2B)<sup>16-21</sup>. Subsequent binding events are governed by a partitioning parameter ( $K_x$ ). This model of multivalent engagement successfully represents other receptors with a similar binding configuration such as FcεR and TCR<sup>18,21</sup>. A critical extension of this model that we made when applying it to the FcγR family is extending it for multiple receptors present. In doing so,  $K_x$  is proportional to the affinity of the receptor, which is necessary for the model to follow thermodynamic laws (detailed balance)<sup>1</sup>.

The largest hurdle to applying this model for IgG mixtures is performing the binding configuration calculation. Though the number of microstates explodes in a combinatorial manner (we have to integrate over all possible mixtures of IC binding states—e.g., an IC bound at site 1, bound at site 1 and 2, etc—weighted by their individual likelihood), modeling the probabilities of these states as a branching process ensures we can efficiently calculate the macroscopic binding we expect to observe<sup>12</sup>. Importantly, despite many more possible binding configurations, there is no additional parametric uncertainty relative to our published model<sup>1</sup>. Indeed, because of this, we do not necessarily need to fit any new parameters and can directly make predictions based on our published parameterization (fig. 2). As previously,  $K_x$  must be proportional to the affinity of an interaction to satisfy detailed balance, and so there is only one  $K_x^*$  value we need to fit ( $K_x = K_x^* K_a$ )<sup>1</sup>. We will nevertheless perform fitting to this new

data, however, in case it can provide more exact parameterization for  $K_x^*$ .

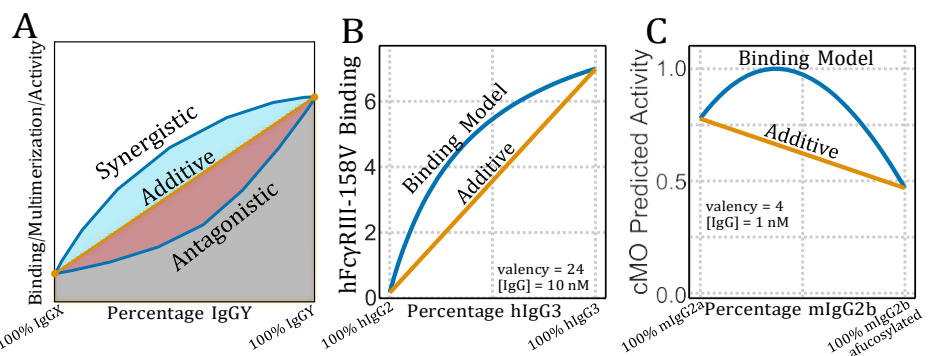
### 1.3. Fit and verify that the model can predict effector response *in vitro*

As validation of our model, predictions of binding and effector response will be evaluated in peripheral blood mononuclear cells (PBMC). PBMCs will be separated and differentiated into individual effector cell populations and stimulated with the same IC complexes<sup>1,32,35-37</sup>. As before, we will assume effector response is proportional to the amount of activating receptor minus the amount of inhibitory receptor found in bound complexes of multiple receptors<sup>1</sup>. This construction satisfies the criteria that increases in activating or inhibitory receptor multimerization have their expected effect, and that multimerization is essential for FcγR

activation<sup>1</sup>. Though this is the most parsimonious construction that satisfies these rules, we will keep in mind this assumption and can easily explore alternative constructions.

Effector cell response will be quantified through induced cell type-specific cytokine secretion since we are stimulating with synthetic ICs (e.g., we cannot measure ADCC)<sup>32</sup>. Ten IC complex mixtures with large predicted variation in response, comprised of the isotypes and glycosylation forms from Aim 1.1, will be selected to test with each cell type. We will focus on monocytes, macrophages, and NK cells due to their ease of isolation and role of cytokine secretion during effector response<sup>35</sup>. The FcγR expression of these populations is well-characterized, but we can re-quantify this if needed using our well-established protocols<sup>1,3,38</sup>. We will measure a panel of >5 cytokines (including IL-6 and IFNγ) in parallel by bead-based ELISA to ensure our results are not cytokine specific and our ranking is similar across all IC-responsive cytokines within a cell type<sup>32,35</sup>. As different cell types might have very different sensitivities to activated FcγRs, we will test our model's predictive capacity by comparing predicted and actual *ranking* (Spearman correlation) of response strength to these 10 mixed ICs within a cell population. We expect close agreement between the IC compositions predicted to maximally induce a response in each cell population, and the responses measured. In addition to measuring cytokine secretion we will also study the phagocytosis of fluorescently labeled (FITC coupled TNP-BSA) IC. By using a combination of intracellular and extracellular FACS staining for FITC we will be able to distinguish between cell surface bound and intracellular antigen. As a second independent verification of IC phagocytosis of fluorescently labeled IC we will use cyto-spins of IC fed monocytes and macrophages and analyse intracellular IC via immunofluorescence microscopy. This work will demonstrate that a binding model can predict which effector cell populations will respond to ICs of mixed composition, helping to make sense of the vast number of possible IC compositions.

Finally, to validate use of this model for mIgG interventions in Aim 3, we will test a small subset of mixed ICs for our ability to predict relative effector response. From previous work we have both affinities and receptor abundance measurements with which to make model predictions<sup>1</sup>. We will select five mixtures of mIgG1/2c, with large variation in their predicted response, to test (see Aim 3.1). The same effector populations as above will be isolated from mouse spleens, and cytokine response measured by bead-based ELISA. We will test agreement of our measurements and model predictions with the same strategy as above.



**Figure 3: Strategy for evaluating synergy, and preliminary evidence a multivalent binding model predicts non-additive interactions.** A) We expect that, when plotting a quantity from the binding model and varying the relative abundance of two IgG Fc compositions, the output quantity would have a linear relationship with mixture composition. We take deviations from this relationship to be either synergy or antagonism depending on whether they are above or below the line, respectively. This outcome will be summarized by quantifying the area under the curve for both the additive and actual case ( $S = (A_{\text{actual}} - A_{\text{additive}})/A_{\text{additive}}$ ). B) Example of predicted synergy in IC binding. C) Example of predicted synergy in murine classical monocyte activity (receptor expression previously measured<sup>1</sup>).

**Preliminary Data** All of the methods used in Aim 1 are demonstrated in previous studies from our labs<sup>1,32</sup>. As exemplified in fig. 2, we have successfully measured binding in the panel of CHO cell lines used here and demonstrated that a multivalent binding model can account for binding of ICs comprised of single IgGs. This work successfully predicted *in vitro* and *in vivo* effector response both for human and murine ICs.

In preliminary work we have implemented the most basic components of the multi-IgG binding model to demonstrate that calculating binding with this approach is indeed feasible (fig. 3). To verify correctness we have compared this new implementation to our published model for cases of a single IgG present and see agreement. The examples of non-additive interaction also fit intuitively with cases of synergy and antagonism we expected to observe. In fig. 3B, hIgG2 has almost no binding, while hIgG3 is a high affinity interaction. Sweeping between each IgG therefore is, in effect, changing the valency of the IC, and the first few hIgG3 added to the left of the plot have the greatest effect on the avidity of the interaction. One would expect adding a second binding site to have a larger relative avidity effect than adding a sixth site, like this suggests. In total, while additional work is necessary to make this binding model implementation usable for these studies, we do not expect challenges.

**Challenges & Alternative Approaches** We have previously used all of the methods in Aim 1 and so do not anticipate significant challenges in these experiments. If our binding measurements do not match our modeling predictions, we will first investigate whether the discrepancy is in a subset of the measurements (e.g., those with a certain IgG), specific outliers, or across all the data. The hFcγRIIIA-158F/hIgG4 case in fig. 2 provides an example, where we identified an outlier and traced it to the underlying affinity measurements<sup>1</sup>. If there are discrepancies across a subset of the data, we will investigate the underlying molecular mechanism. We can also use the single IgG measurements from our published work as a guide for whether discrepancies are modeling or experimental problems.

If our measured and predicted binding is consistent but our cell response measurements do not match our predictions, this will provide an opportunity to investigate additional mechanisms of effector regulation beyond binding. For example, recent reports have implicated clustering of hFcγRI as a mechanism of inside-out signaling<sup>39</sup>. If we observe divergent results from what we predict, we can investigate whether mechanisms such as inside-out signaling influence the relative response to different ICs. We can use a panel of blocking antibodies targeting each FcγR to isolate the influence of each FcγR expressed within a cell population<sup>2,38</sup>. Importantly, these other regulatory factors can contribute to effector cell response only after a cell has interacted with an IC, and so IC binding should still be a dominant factor in effector cell response, and certainly one for which we must first account.

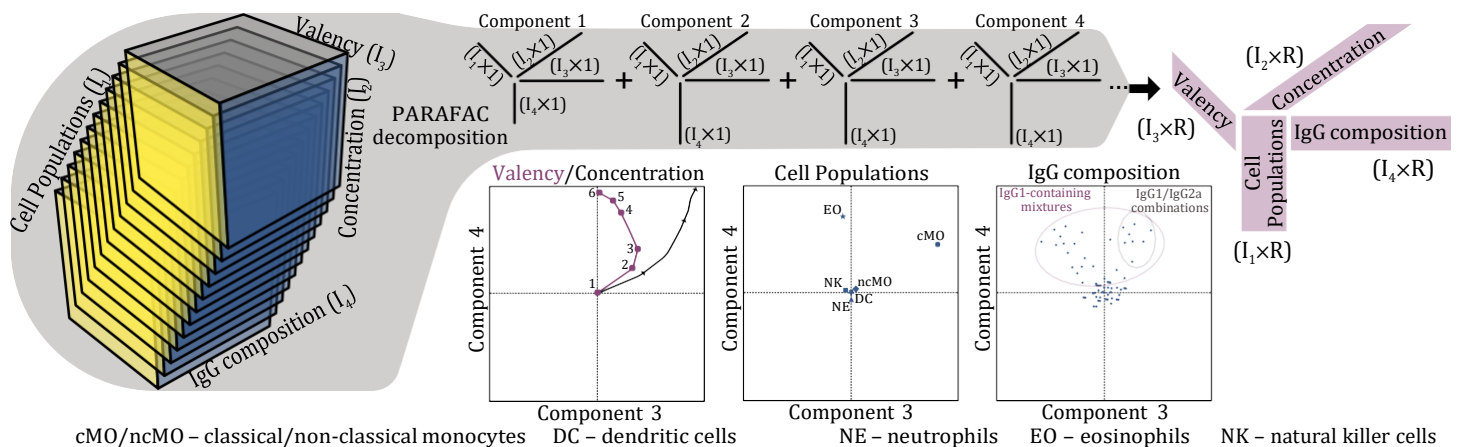
## **Aim 2: Map human and murine IgG isotypes to one another according to conserved effector response**

**Rationale** Unclear homology between the human and murine FcγR families stymies our ability to translate findings from murine models of disease<sup>11</sup>. Using the overarching hypothesis that there is conserved regulation at the level of cell type-specific effector response, we will use our interaction model to build a homology map between species.

**2.1. Map IC composition to effector responses by tensor decomposition** An inter-species map would greatly aid translation of findings in murine models of IgG-related diseases and IgG-elicited cell killing. Assembling such a map first requires a global view of how the family is regulated. Our model of FcγR engagement, besides fit parameters common to all FcγR-IgG pairs, requires affinities for each FcγR-IgG pair and the profile of FcγR expression within a cell. As these exist for effector cell populations within both murine and human cells<sup>1,38,40</sup>, we will use these to assemble a data compendium of the predicted effector responses across IgG combinations in each species.

All combinations of IC concentration and composition will be varied to create a data tensor of model-predicted FcγR activity within each effector cell population. We will use the same constructed activity calculation as in Aim 1, as it successfully predicts effector response in our previously published work and will be further validated in Aim 1.3. We will simulate every possible combination of cell population (eosinophils, NK cells, dendritic cells, neutrophils, classical monocytes, and non-classical monocytes based on their FcγR expression), valency (1-26), concentration (log-scaled, 1 fM-1 μM), and IgG composition (combinations of 1:1, 1:2, 1:5, and 1:10 mixtures). We will start with the FcγRIIIA-158F





**Figure 4: Overview of tensor factorization to map effector response.** A central challenge in engineering IgG-elicited cell killing is that any intervention is pleiotropic on multiple levels. That is, an IC can have differing effects based on its IgG composition, concentration, and valency, with responses from distinct cell populations. With a binding model we can predict the outcome of any individual combination of factors but still have trouble mapping and visualizing the predominant axes of variation in these data. Through tensor factorization, this four-dimensional space can be decomposed into component factors that capture predominant axes of variation. For example, in the hypothetical factorization results shown, components three and four increase with concentration, and valency increases along component four. Component three, however, shows a bimodal valency relationship. Eosinophils and classical monocytes are activated along component four, while only the latter are activated along component three. Through the last components plot, we can see which mixtures drive movement along each component; IgG1-containing mixtures are positively associated with component four, while component three is exclusive to IgG1/IgG2a combinations. So, if we want to maximally activate eosinophils, these plots indicate we want a combination with IgG1 and higher valency. In this way, factorization provides a design schematic for variation in effector response.

and FcγRIIA-131R genotype; however, this method also provides an opportunity to look at predicted differences in regulation based on genotype in future studies. While we are starting with a selected set of effector cell populations for which we have existing FcγR abundance measurements, these results can quickly and easily be updated with new measurements (e.g., macrophages, dendritic cell subsets). These ~100,000 values for each species capture the variation in predicted effector function due to differences in binding propensity to each cell population but remain challenging to interpret due to the high-dimensional nature of the data.

We will then utilize canonical polyadic (CP) decomposition to visualize these data, a data reduction technique similar to principal component analysis in some aspects, representing the variation in effector response in a reduced dimensionality space<sup>14</sup>. Briefly, this method finds factors, or directions of variation in the data, and the relative contribution of each variable to that factor. The parallel plots of each factor and the variables involved are essentially a map of the variation present within the data. Importantly, with a sufficient number of factors, the data tensor can be perfectly reconstructed from the factors, and so information is preserved in the factorization process. We will determine the number of factors necessary to capture >95% of the variance in predicted response for each cell population upon reconstruction. This factorization will then be used as a “map” for regulation of the FcγR family.

**2.2. Correlate murine and human effector responses according to similar cell population response** We hypothesize that, while the individual FcγRs/IgGs are not directly conserved, there exists conserved regulation in the form of which cell populations are activated coordinately. That is, we can identify cross-species IC pairs targeted to have the same cell population responses. To do so, we will correlate each component of the cell population factorization between species. Identifying significant correlations here (Pearson correlation, with family-wise error rate correction through cell population randomization) will test our hypothesis that conserved regulation exists targeting the same cell populations.

As each resultant component of the factorization represents a separable subset of variation in FcγR/IgG regulation, we expect to observe one-to-one correspondence between components of the human and murine factorization. Therefore, we expect to see that each component of the cell population factorization has a single significant correlation pair (fig. 5).

### 2.3. Verify this mapping predicts known, similar cross-species effector cell responses

Experimentally testing our mapping from Aim 2.2 would require isolation of many immune populations from both murine and human sources, along with cytokine measurements and functional characterization in both species. This scale of validation is outside the scope of this proposal but will be enabled in future studies by the present analysis. However, we will check for a few features we expect to observe: (1) hlgG1/hlgG3 and mlgG2a generally have greater effector responses and are more pro-inflammatory. We expect to identify linked components that involve mixtures of both these IgG<sup>11</sup>. (2) Both human and murine families have a single inhibitory receptor with similar expression patterns, hFcγRIIB/mFcγR2B, and so we expect that these will be aligned in the factorization (weighted similarly in linked components)<sup>11</sup>. (3) hFcγRIIIA/mFcγRIII are the sole Fc receptor on NK cells. Therefore, we expect to find linked components that represent activation of these receptors and includes NK cell response<sup>11</sup>.

In addition to the individual component-specific relationships we expect to find above, we will test our ability to apply our homology model for “translating” between murine and human IgG compositions in Aim 3.3. This will evaluate the most translationally-valuable aspect of our results here.

**Challenges & Alternative Approaches** Importantly, while Aim 2 aids translation of Aim 3 and addresses a fundamental question about conservation of this receptor-ligand family, execution of both can proceed independently. As CP decomposition efficiently and parsimoniously captures variation in the original data tensor, we expect the factors identified to clearly display any correspondence between species. However, if we do not identify components with corresponding variation between species, we can take a more targeted approach. With an IC composition that leads to a certain set of predicted effector cell response in humans (e.g., NK cells, but not other cell types), we can then vary murine IC composition to look for compositions that have a matching profile in mice (or vice versa). This will still identify homology across species.

We expect CP decomposition to be the most useful and easily interpretable method for tensor factorization, given that it provides parallel components along each dimension. However, many other factorization methods exist which may have benefits depending upon the variation found in the data. For example, Tucker decomposition is a more flexible generalization of CP decomposition that allows for linking between components through a core tensor<sup>41</sup>. This creates a tradeoff of fewer components being necessary to explain the data, but additional challenge in visualizing the core tensor. Additionally, both CP and Tucker decomposition can be forced to have only non-negative components. By constraining the factorization in this way, this often makes the resulting components much easier to interpret (since it separates out balanced negative and positive effects)<sup>42</sup>. In total, there is a rich toolbox we can apply to further explore the data here to create an effective map of IgG-FcγR regulation.

### Aim 3: Link IgG effects and *in vivo* efficacy to identify & verify synergistic IgG-elicited cell killing

**Rationale** Successfully identifying examples of synergistic effector-elicited killing will demonstrate that IgG isotypes have unique properties in combination. Moreover, it will show that a binding model can successfully identify these cases to engineer response and that this synergy can be employed successfully *in vivo*.

|                   |   | Human Components |   |   |   |   |
|-------------------|---|------------------|---|---|---|---|
|                   |   | 1                | 2 | 3 | 4 | 5 |
| Murine Components | 4 |                  |   |   |   |   |
|                   | 5 |                  |   |   |   |   |
|                   |   |                  |   |   |   |   |
|                   |   |                  |   |   |   |   |
|                   |   |                  |   |   |   |   |

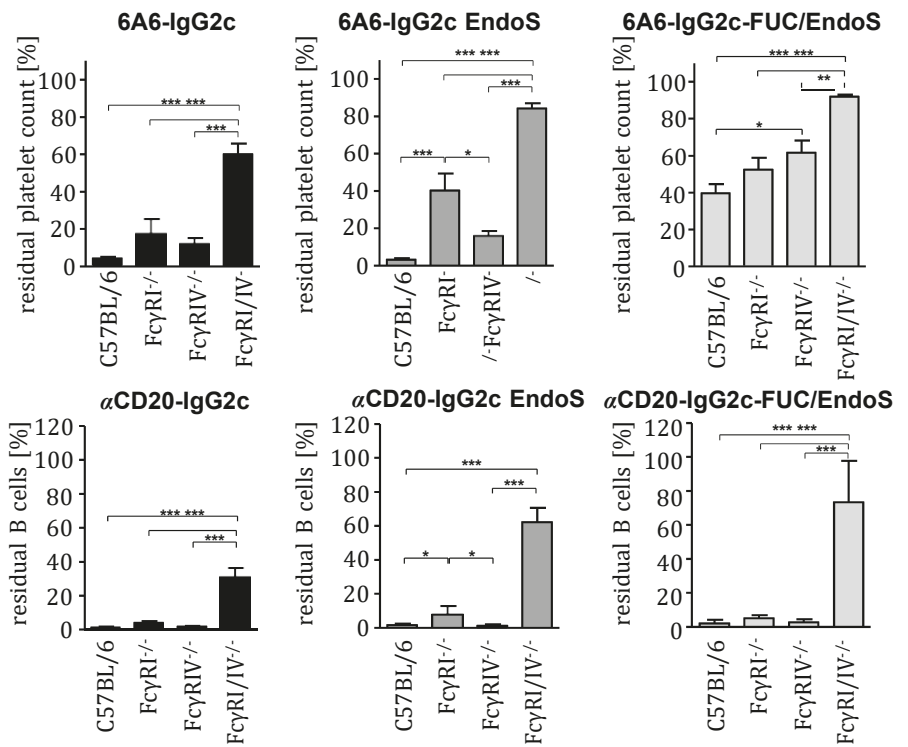
**Figure 5: Schematic for expected results of human-murine cell population correlations.** We expect to observe one-to-one links between each component of the factorization across species. However, this need not be the same component number in each species. For example, here, murine component three and human component two might be correlated due to shared weighting of classical monocytes and NK cells.

**3.1. Regress single IgG treatments and *in vivo* cell clearance to identify synergistic combinations** We will use a passive mouse model of immunothrombocytopenia (ITP) and a model of cytotoxic antibody-mediated B cell depletion (CD20 specific) as model systems for IgG-elicited cell killing<sup>43</sup>. These model systems have beneficial properties, including that effector cell-elicited killing (platelet or B cell depletion) can be assessed and quantified rapidly, and do not involve long-term inflammation with unknown compensatory changes. Moreover, the responsible effector cells are liver resident Kupffer cells and/or resident monocytes, which have a well-defined FcγR expression pattern<sup>3,4</sup>.

To predict cell killing, we will use a similar regression approach to the one we recently performed for anti-tumor IgGs in a B16F10 melanoma model<sup>1,2</sup>. Briefly, for each intervention, the binding model predicts a level of FcγR activity for each cell population, and then those “activities” are regressed against fractional reduction in cell number (fig. 8)<sup>1</sup>. This regression will be performed using a  $y = 1 - \exp(-X \cdot p)$  relationship, where  $X$  is the matrix of activities and  $y$  is the fractional reduction in cell number. This construction corresponds to an exponential survival distribution and thus an underlying random process wherein every cell is at uniform risk of clearance. The structure of the regression portion of the model is unchanged by considering mixed composition ICs; therefore, with our updated binding model we will be able to immediately make predictions about *in vivo* response after fitting. Like with the B16F10 model, we will use a panel of pre-existing experimental results in which different IgG isotypes, glycosylation variants, and FcγR knockouts have been evaluated. A wider panel of these experiments exists for both the ITP and CD20-depletion models than the B16F10 case, in fact, which will aid exact parameterization of the model and therefore accurate predictions (e.g., fig. 6)<sup>2,43,44</sup>. Model prediction will be quantified through leave-one-out (LOO) and leave-one-isotype-out crossvalidation. The significance and distribution of derived quantities will be estimated by bootstrap<sup>45</sup>.

We will identify a predicted case of synergistic interaction between mIgG isotypes and/or glycosylation variants (fig. 7) for each target cell. Synergy will be calculated according to the Bliss independence rule<sup>23</sup>. That is, in the absence of synergy, we will assume each antibody has an independent, proportional decrease in the number of platelets or CD20-positive cells observed. IgG combinations with the greatest predicted reduction over that from an additive effect will be selected. If these cases are widespread we will also consider the disease relevance of the glycosylation and/or isotype combination (e.g., prioritize fucose/non-fucose combinations due to their therapeutic manufacturing relevance). We also expect the combinations identified will be comprised of mIgG1 and/or mIgG2c, given the efficacy of these as single agents<sup>2,43,44</sup>.

Note that this approach is not limited to synergy in cell killing arising through synergy in FcγR binding (e.g., fig. 3C). For example, high affinity IgGs tend to also have a higher affinity for the inhibitory hFcγRIIB/mFcγR2B. However, these higher affinity IgGs could play an outsized role on initial monovalent binding of ICs. ICs with a small number of high-affinity IgGs along with many lower affinity (but mFcγR2B non-binding) IgGs therefore might lead to greater activation than ICs with either IgG alone.



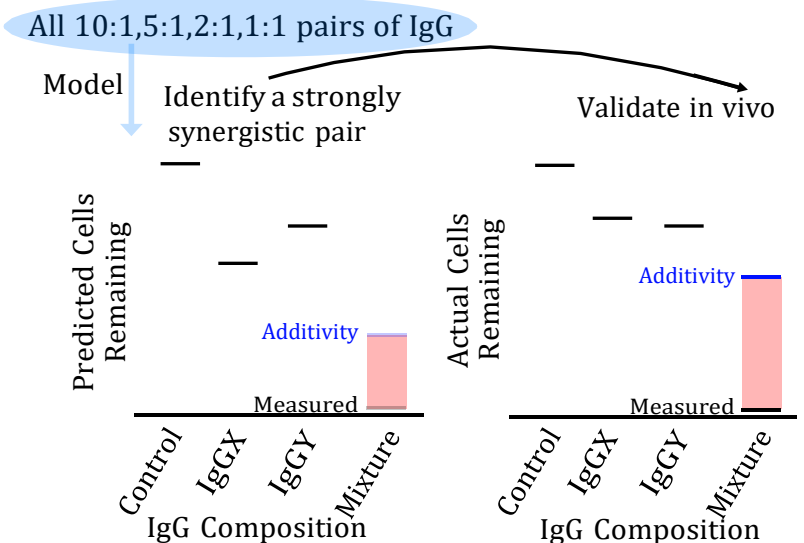
**Figure 6:** Both platelet and B cell IgG-mediated depletion are FcγR-, Fc isotype-, and Fc glycosylation-dependent<sup>43</sup>. Quantification of platelet and B cell depletion using either 6A6 or anti-CD20 IgG, respectively.



**3.2. Verify predicted cases of synergistic effector response *in vivo* within models of antibody-dependent, effector-mediated platelet and B cell depletion** All four human and mouse IgG isotype variants are available for both *in vivo* model systems<sup>2,43,44,46,47</sup>. Afucosylated IgG isotype glycovariants can be generated by recombinant antibody production in LEC13 cells as we have done before<sup>48</sup>. The selected combinations will be evaluated in C57BL/6 mice in parallel to matched treatments with either IgG alone. Platelet and B cell depletion in the blood will be assessed before, 4 hours after, and 24 hours after the corresponding IgG isotype mixture injection by FACS analysis. We will use 8 mice per treatment, or 32 total (control, each IgG alone, and the combination), to provide sufficient power (0.8) for each test below.

In both target cell models we will evaluate the outcomes of the experiment in a few different ways. First, to evaluate the predictive capacity of our model in this independent cohort, we will test that there is significant correlation between the predicted and actual target cell depletions (Pearson correlation). Second, we will test that (1) the deviation observed with the combination is toward synergy as predicted, and (2) the synergy is statistically significant (mixed effects model, significance tested by bootstrap). Within Aim 3.3 we will additionally test the mechanism by which synergy arises. In total, this work will demonstrate that mixtures of IgG have unique properties of effector-elicited cell clearance in combination.

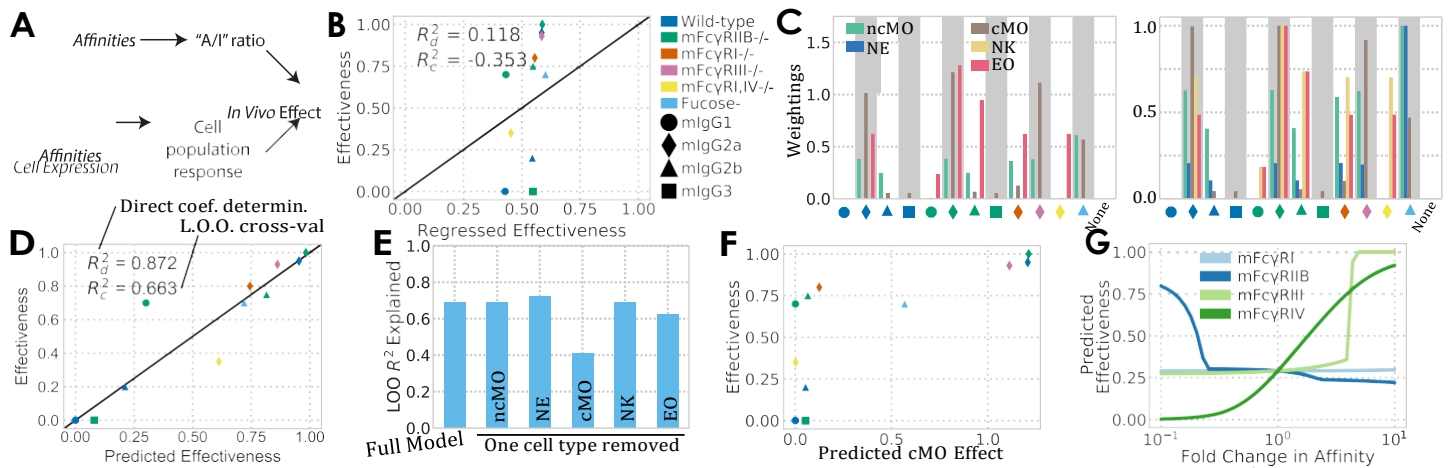
Lastly, to test our predictions of homology between the human and murine FcγR families, we will test these combinations in humanized mouse models as we have done before<sup>46,47</sup>. All single and mixture conditions used above will be “translated” from murine to human IgG compositions using the results of Aim 2. These 6 conditions (3 interventions for either B cell or platelet targeting) will be quantified for target cell depletion at the same times as above. We will test for significant correlation between the results from the C57BL/6 and humanized models (Pearson correlation). This will serve as partial validation of the modeling in Aim 2 and demonstrate the value of the human-mouse homology model.



**Figure 7: Synergy strategy.** We will verify our cases of predicted synergy through: (1) correlation between prediction & response, (2) significant synergy in the direction predicted, and (3) mechanism of synergy (Aim 3.3).

**3.3. Identify the relevant cell populations and mechanisms of synergistic effector response** Broadly, there are four possible underlying mechanistic sources of synergy/antagonism between IgGs: (1) at the level of binding to an individual effector cell population, (2) not in binding, but in the resultant response of an individual cell population, (3) additivity on previous levels, but with two IgGs targeting a differing complement of cell populations, or (4) additivity across cell populations, but some other emergent interaction through cell communication.

To resolve the underlying mechanisms of interaction, we will use two initial experiments. First, we will use TNP-BSA binding studies as outlined in Aim 1, with primary effector cell populations, to identify whether mechanism (1) might explain the interaction we observe. Individual effector cell populations will be separated and then incubated with each IgG separately, or with the indicated mixture of both IgGs. Significant interactions between the IgGs in binding will be quantified through deviation from additivity. Second, we will quantify the isobologram of ADCC/ADCP effector response in each cell population to address mechanism (2). For B cells, we will incubate each population with B cells and anti-CD20 IgGs of the same mixtures as those tested *in vivo*. The number of remaining B cells will be quantified through B220 staining after 72 hours incubation<sup>44</sup>. For platelets, we will incubate them with each cell population and 6A6 antibodies of the same Fc compositions. The number of remaining platelets will be quantified at 24, 36, and 72 hours of incubation using calcein staining then flow cytometry. Finally,



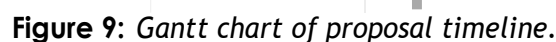
**Figure 8: An FcγR-IgG binding model accurately predicts in vivo IgG-mediated tumor cell killing<sup>1</sup>.** A) Schematic of earlier IgG isotype experiments (top) and our approach (bottom). In earlier work, the ratio of the highest affinity activating receptor to that of the inhibitory receptor (A/I ratio) was proposed to predict response<sup>2</sup>. B) Effectiveness (proportional reduction in lung metastases, i.e. no reduction is 0.0, complete is 1.0) of individual mlgG interventions versus the A/I ratio for each mlgG constant region. C) Individual cell activities calculated for each intervention using receptor multimerization predicted by multivalent binding model. Each quantity is scaled according to the weighting applied by the fitted regression model (left) or by maximum cell type response observed (right). D) Predicted versus observed effectiveness. E)  $R^2$  with individual input components removed. F) Calculated activity index for cMO versus overall effectiveness of each intervention. G) Predicted effect of modulating each individual mFcγR affinity of mlgG2b. Regression performed with exponential survival relationship in contrast to published work, due to benefits explained in Aim 3.1<sup>1</sup>. EO: eosinophil, cMO: classical monocyte, ncMO: non-classical monocyte, NK: natural killer, NE: neutrophil.

we will investigate mechanism (3) by determining whether additive combinations of the individual cell population effector response measurements can explain the overall responses we observe *in vivo*.

We strongly expect these first three mechanisms will explain the synergy we observe, as these are the three mechanisms captured by our modeling prediction. Verifying the relevant cell populations involved will further validate the accuracy of our model. Based on our previous *in vivo* studies in both the ITP and B cell depletion model, we would expect that liver resident Kupffer cells and/or resident monocytes are the relevant effector cell populations. Both cell subsets express all activating and the inhibitory FcγRIIB, making it difficult to distinguish the contribution of both cell subsets purely based on using individual FcγR knockout mice. However, by selectively depleting bone marrow derived resident monocytes through small doses of clodronate liposomes<sup>3</sup> or by using a titrated irradiation approach to generate mice with a selective lack of FcγRs on bone marrow derived monocytes or liver resident Kupffer cells<sup>40</sup> we will be able to delineate if B cell or platelet depletion through mixed IgG subclass antibodies behaves differently compared to the use of one IgG subclass *in vivo*. Briefly, animals will be injected with select ratios of IgG subclass mixtures of platelet (6A6) and B cell (CD20) specific antibodies. B cell and platelet counts will be assessed in the blood 4 and 16 hours after antibody injection. To assess if tissue resident Kupffer cells are involved in B cell and platelet depletion, we will inject mice with 10 μL of clodronate liposomes, which shows a rather selective depletion of bone marrow derived resident monocytes<sup>3</sup>. Moreover, we will generate bone marrow chimeric animals selectively expressing activating FcγRs either on tissue resident Kupffer cells or bone marrow derived monocytes by irradiating FcγR deficient or sufficient animals with 6Gy followed by a reconstitution with bone marrow of FcγR sufficient or deficient mice<sup>40</sup>. Should mixed IgG subclass dependent target cell depletion involve other cell populations, we can also study the involvement of NK cells or neutrophils by using either NK- or neutrophil-depleting antibodies or NK cell- or neutrophil-deficient mouse strains. We expect to observe a reduction in the degree to which either platelets or B cells are depleted that is consistent with our model's weighting for that population with the given mixture. Our model treats cell populations as having separable contributions to platelet or B cell depletion. Therefore, if the effects

**Challenges & Alternative Approaches** If IgG combinations selected in Aim 3 do not show synergistic responses, the mechanism-focused measurements in Aim 3.3 will be extremely valuable to diagnose any inconsistencies from the model predictions.

To relate the activities of each effector cell population to depletion of B cells and platelets, we have to define a survival function, or a function for the relative risk of each additional target cell to be cleared. We propose using an exponential distribution, because this corresponds to the outcome observed when each target cell is at identical “risk” of being cleared. However, alternative survival distributions exist, corresponding to underlying definitions of relative risk. In particular, the Gompertz and Weibull functions correspond to multiplicative and additive differences in “risk” among target cells. Therefore, using these distributions could capture, for example, if platelets and B cells lie on a continuum from easy to hard to clear for reasons unrelated to their antigen availability.



## PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved

☐ Yes

☒ No

Is the Project Exempt from Federal regulations?

☐ Yes

☐ No

Exemption Number

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

☐ 6

☐ 7

☐ 8

Does the proposed research involve human specimens and/or data

☐ Yes

☒ No

Other Requested information

## Vertebrate Animals

### 1. Description of Procedures

The proposed studies involve the use of mice specifically in Aims 1.3 and 3. Animals will be housed in the animal facilities of the Friedrich Alexander University (FAU) Erlangen-Nuremberg. The following procedures will be used:

#### Mouse care

Six- to eight-week-old female C57BL/6, FcγRI<sup>-/-</sup>, FcγRIII<sup>-/-</sup>, FcγRIV<sup>-/-</sup>, FcγRI/IV<sup>-/-</sup>, FcRγ<sup>-/-</sup> mice (all C57BL/6 background) will be obtained from commercial vendors (C57BL/6) or from in house breeding stocks (Fc-receptor deficient strains) and will be used as recipient mice for the respective B cell and platelet depleting antibodies. To generate bone marrow chimeric animals, bone marrow recipient mice will be irradiated with 6Gy followed by an intravenous injection of bone marrow from donor animals (C57BL/6 or Fc-receptor deficient mouse strains) to generate mice with deletions of Fc-receptors selectively on hematopoietic cells and not on tissue resident macrophages. In select experiments humanized mice will be generated. For this purpose, new born Nod/Scid/γ<sub>c</sub><sup>-/-</sup>FcRγ<sup>-/-</sup> mice will be irradiated with 1.2 Gy followed by an intravenous injection with 50,000 human hematopoietic stem cells (HSC) 4 to 6 hours after irradiation. HSC are provided by the placenta blood bank from the University of Erlangen-Nürnberg after written consent of patients and according to the ethical guidelines of the FAU. After 3-4 months mice will be analyzed for the presence of human cells in the peripheral blood and positive mice will be used for further experiments. All mice will be housed in IVC cages and will be monitored on a daily basis by professional animal caretakers employed by the animal facility.

#### 6A6 antibody dependent platelet depletion

Mice will be injected intravenously with 0.2 µg/g body weight of the recombinant 6A6 antibody isotype switch-variants or mixtures thereof diluted in 200 µl of PBS. Platelet counts before and 4 hours and 24 hours after antibody injection will be determined by blood collection (20 µl) from the retro-orbital plexus followed by a 1:10 dilution in PBS/5% BSA and a measurement in an Advia 120 hematology system (Siemens). To delete predominantly blood derived resident monocytes, mice will receive an intravenous injection of 10 µl of clodronate liposomes. In humanized mice 6A6 humanized antibodies will be used at 0.5 µg/g body weight.

#### CD20 antibody dependent B cell depletion

B cell depletion will be induced by intravenous injection of 5 µg/g of CD20 specific IgG subclass switch variants or mixtures thereof. B-cell counts will be determined in the peripheral blood before and 6 or 24 hours after antibody injection by FACS analysis. To delete predominantly blood derived resident monocytes, mice will receive an intravenous injection of 10 µl of clodronate liposomes. To study a potential involvement of NK cells or neutrophils we will inject mice one day before administration of the CD20 specific antibody with 250 µg of a Ly6G specific antibody (clone 1A6) to deplete neutrophils or with 100 µg of the NK cell specific NK1.1 antibody to deplete NK cells. In humanized mice, human CD20 specific isotype switch variants of Rituximab will be used at 5 µg/g body weight.

### 2. Justifications

There are no alternative models to the use of mice for the functional analyses IgG/immune complex activity. The experiments proposed in Aims 1.3 and 3 are critical to assessing the efficacy of homotypic or heterotypic IgG immune complex activity in the clinically most meaningful settings. Finally, NSG mice are well-characterized mouse strains for the transplantation of a human immune system. We have made every effort to use the minimum number of mice needed while maintaining statistical power in our results.

### **3. Minimization of Pain and Distress**

Every effort has been made to use the absolute minimal number of mice in any one experiment and experimental design has been optimized make use of common controls where feasible, to reduce the number of mice needed on a per-experiment basis.

Staff will ensure regular observation of the mice during experimental interventions, and if any mouse experiences any of the below listed signs and symptoms, the mice will be immediately euthanized.

- Anorexia or persistent dehydration
- Weight loss of 15% over 72 hr period or failure to gain weight in young animals
- Extreme difficulty in remaining upright or moving
- Experiencing muscle atrophy or emaciation
- Is lethargic or does not respond to gentle stimuli
- Becomes hypothermic
- Is unconscious or in a coma
- There is bloodstained discharge emanating from any orifice
- Trouble breathing
- Exhibits anemia
- Is experiencing abdominal distension
- Has trouble with incontinence or experiencing diarrhea

Weekend, holiday and emergency care is provided through daily animal health checks by technicians, providing husbandry as needed and notifying investigators and/or the consulting veterinarian of any animal health problems. The attending veterinarian, back up veterinarian, Animal Facility Associate Director and Veterinary Technologists are available at all times via cell phone. The Animal Facility has an Emergency Response Plan that is reviewed and updated yearly by the Safety Office, Plant Operations and the Animal Facility Office.

### **4. Method of Euthanasia**

Animals will be euthanized with carbon dioxide or by cervical dislocation under previous anesthesia with isoflurane (according to German rules and regulations and consistent with AVMA guidelines).

## **Multiple PI Leadership Plan**

Dr. Meyer (UCLA) and Dr. Nimmerjahn (FAU) will provide oversight of the entire project including development and implementation of all policies, procedures and processes. Both PIs will share responsibility for implementation of the specific aims as described in the Research Strategy. Together they will oversee the scientific research, analyze and interpret research data, report results to the scientific community, and disseminate approaches, methods, models, software, and tools. They will also ensure that systems are in place to guarantee institutional compliance with US laws, DHHS and NIH policies including biosafety, human and animal research, data and facilities.

Dr. Meyer will serve as the contact PI and lead for the computational modeling and data analysis aspects of the project. He will be responsible for reporting progress to the NCI Program Officials on the project's research through a structured reporting format. Dr. Nimmerjahn will serve as the lead for the *in vitro* and *in vivo* experimental measurements. Drs. Meyer and Nimmerjahn will communicate regularly, either by phone, e-mail, or in person, to discuss experimental design, data analysis, and all administrative responsibilities. They will work together to discuss any changes in the direction of the research projects and the reprogramming of funds, if necessary. A publication policy will be established based on the relative scientific contributions of the PIs and key personnel. Both Drs. Meyer and Nimmerjahn have experience in managing highly collaborative work involving contributions from multiple individuals. Indeed, their recent joint publication is demonstrated success from their collaboration.

Drs. Meyer and Nimmerjahn will meet regularly with the staff and trainees committed to this project to evaluate scientific progress, troubleshoot experimental difficulties, decide experimental directions and mediate any conflicts that occur. In addition, there will be monthly meetings where all members of the project will meet to share information, solve technical issues and ensure that the timeline and milestones are achieved.

### ***Intellectual Property***

The PIs will grant necessary access rights to the pre-existing patents and/or the patents potentially generated within the frame of this project for the purpose of this research project to all the other PIs and key personnel on a non-exclusive royalty-free basis. Each PI shall take appropriate measures to ensure that he/she can grant these access rights. Any pre-existing intellectual property will remain the property of the party that created and/or controls it. An Intellectual Property Committee composed of representatives from each institution that is part of the grant award will be formed to work together to ensure the intellectual property developed by the PIs is protected according to the policies established in the agreement.

### ***Conflict Resolution***

If a potential conflict develops, the PIs shall meet and attempt to resolve the dispute. If they fail to resolve the dispute, the disagreement shall be referred to an arbitration committee consisting of one impartial senior executive from UCLA and FAU as well as a third impartial senior executive mutually agreed upon by both PIs. No members of the arbitration committee will be directly involved in the research grant or disagreement.

### ***Change in PI Location***

If a PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement at one of the participating institutions.



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## **Consortium/Contractual Arrangements**

We have chosen UCLA as the primary institution because Aaron Meyer (co-PI) is directing the modeling and data analysis, and integration of these with experiments across the project. These tasks encompass all aims of the proposal and are key to interpreting and later directing the experimental efforts. Dr. Meyer has a long-standing collaboration with the other co-PI Falk Nimmerjahn, including joint publication. Dr. Meyer will take primary responsibility for communications with the NIH and other administrative functions. With that said, the proposed project is a close collaboration that brings together joint expertise in immunology of effector response, receptor signaling, modeling, and data analysis, as represented by the co-PI's at Friedrich-Alexander-Universität Erlangen-Nürnberg and UCLA.

The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the agency's consortium agreement policy and are prepared to establish the necessary inter-organizational agreements consistent with that policy.

## **Resource Sharing Plan**

The investigators of this proposal are aware of and agree to abide by the principles for sharing research resources as described by the NIH in "Final Statement on Sharing Research Data" [<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html>] and in the application RFA. Resources developed within the scope of this application will be made fully available to the scientific community. This includes publications (through PubMed Central and/or publication in open-access journals), raw data, and reagents. All plasmids used in the course of the study will be made available through a repository such as AddGene. Mass spectrometry data will be deposited in a repository such as PRIDE. The associated source code for all analyses will be made available in full on or before publication. Other forms of raw data will be made available alongside our analysis code and deposited in ImmPort before publication. We do not anticipate the development of any model organisms from this study, and this study does not involve generation of GWAS or genomic data.

## Authentication of Key Resources

**General.** Largely standard laboratory agents will be used and purchased from established commercial sources, i.e. Thermo-Fisher, Sigma-Aldrich, Tocris, Abcam, etc.

**Cell Lines.** We have and will continue to use conventional short-tandem repeat (STR) assessments as our gold-standard for ensuring the provenance of each individual model. This will be performed regularly (at least annually) for each cell line model during their use. Mycoplasma testing will also be carried out periodically (at least every six months).

To verify stable Fc $\gamma$ R expression, we will quantify the absolute abundance of each receptor in each CHO cell line prior to binding experiments. These measurements are performed using by staining with FITC-coupled Abs directed against Fc $\gamma$ RIA (CD64; clone 10.1; BD Pharmingen), Fc $\gamma$ RIIA/IIB (CD32; clone 3D3; BD Pharmingen), and Fc $\gamma$ RIIIA (CD16; clone 3G8; BD Pharmingen). Absolute quantitation is obtained by comparison to a panel of beads with defined numbers of antibody binding sites in each experiment.

**IgG Expression.** We will perform MALDI-TOF analysis to verify the glycosylation profiles of each IgG, as we have done previously (Lux et al, 2013). We will verify (1) that there are no qualitative changes between batches of expressed IgG, and (2) that the expected changes occur in the glycosylation profiles we observe with either EndoS or PNGaseF treatment.

**TNP-IgG Complexes.** To ensure TNP-IgG complexes are formed with the expected differences in valency the complexes will be subjected to polyethylene glycol (PEG 6000) precipitation, as we have performed previously (Lux et al, 2013). We expect and will verify that this shows qualitatively similar differences in molecular weight between TNP-26-BSA complexes, TNP-4-BSA complexes, and free IgG.

**Antibodies.** All antibodies used in the proposed studies are reagents we have previously used and validated in published work (referenced throughout the proposal). All antibodies will be aliquoted upon receipt and stored at  $-80^{\circ}\text{C}$  for long-term storage. Lot number for all antibodies will be logged as part of the experimental records. Where noted, matched knockout or antigen-absent controls are used to verify the antibody specificity within each experiment.

**Data Analysis and Archive.** All raw and processed data is backed up and stored in a versioned manner on network attached storage. The remote backup device is additionally backed up off-site. This arrangement ensures all data is retained, and that a permanent record of modified data is kept. To ensure reproducibility, in-house analysis routines are stored using version control with the entire pipeline of analysis from raw data to analysis products documented. The entirety of this source code and analysis pipeline will be made publicly available with or before publication of its results.

**Mice.** All employed transgenic mouse lines have been previously characterized and described in peer-reviewed publications. Genotype as well as transgene expression and its pattern will be monitored through each generation using standard techniques (PCR, *in situ* hybridization, and immunohistochemistry). Each mouse line will be continuously backcrossed to C57BL/6 to maintain genetic stability. Additional breeders will be obtained from established repositories such as Jackson Laboratory and Mutant Mouse Resource and Research Center. Spermatozoa of non-commercially available mouse lines will be cryopreserved for rederivation in case of genetic instability and natural disasters. Each mouse will be ear-tagged; genotype records will be printed and filed for each mouse identified.