NIH Institutional Biosafety Committee Minutes
Location: Bethesda Main Campus
June 04, 2025
2:00 PM – 5:00 PM
Virtual via Microsoft Teams

Members present
Quorum met: Yes

Harry Malech, Chair, clinician	Stephen Denny, animal research expert	Alicia Alexion, unaffiliated member
Richard Baumann, BSO	Stephanie Goff, clinician, virology	Michael Kujawa, ABSO
Bridgette Billioux, clinician, virology	Althea Treacy, biorisk mgmt.	Luz Angela Rosas, laboratorian
Robert Craigie, recombinant and molecular techniques	Steve Whitehead* recombinant and molecular techniques, virology	

*Provided comments *in absentia*

Guests present

Rebecca Anderson	Hanna Kim	Hong Ha Rosa Nguyen
Theresa Bell	Ryan Leighton	Raluca Semeniuc
Avijeet Chopra	Andrew Mammen	Nirali Shah
Alice Fike	Andrew Martin	Julie Thompson
Kane Kaosaard	Tandees Najimi	Westley Weiss

Announcements & Call to Order

The meeting was called to order by Dr. Malech at 1:02 PM.

Review of the Past IBC Meeting Minutes

07 May 2025 Minutes

a. Comments on minutes, as applicable
b. The minutes were unanimously approved with minor modifications.

New Committee Business

I. BSO preliminary registration approvals since the previous meeting

a. Pathogen registrations: 25-BMC-097, -099
b. rDNA and rDNA/pathogen registrations: 25-BMC-096 (Ho)- III-D-3-a, III-D-4-a- Development of Immunotherapies for Cancer: CAR-T cells, CAR-Macrophages, Immunotoxins, and Bispecific Antibodies (Consolidation). This registration updates and replaces the following registrations previously approved by the IBC: RD-15-V-09, RD-14-IX-18, RD-21-VIII-03, RD-22-XII-12, RD-23-V-08, PRD 6444, PRD 6802, PRD 7330, and PRD 8187. 25-BMC-098 (Kim)- III-E-1- Mammalian cell culture expression of antioxidant proteins such as methionine sulfoxide reductase A, B1, B2, and B3. 25-BMC-100 (Inglese)- III-E-1- Mammalian cell culture with chemical probes
c. Registration amendments: Approved RD amendments ('amend 250604') for the Minutes: RD-22-IX-06 (amend-Greten). RD-23-VII-03 (amend-Pierson). RD-20-IX-11 (amend-Brooks). RD-12-II-05 (amend-Dunbar). RD-21-X-02 (amend-Pierson). RD-24-II-26 (amend-Terabe). RD-21-X-10 (amend-Young). RD-21-IX-03 (amend-Greten). 25-BMC-022 (amend-Hope). RD-19-VI-04 (amend-Muppidi). RD-16-XII-13 (amend-Muppidi). 24-BMC-020 (amend-Mughal), 25-BMC-012 (amend-Katzelnick). RD-

23-I-05 (amend-Ambudkar). RD-18-X-09 (amend-Gonzalez). RD-16-I-11 (amend-Michaelides). 24-BMC-134 (amend-Chiorini). RD-19-X-07 (amend-Seder)

d. Committee discussion: None

e. The BSO asked if there were any questions or concerns with the preliminary approvals. Hearing none, and following the formal review and discussion at the meeting, the IBC concurs unanimously with and formally approves the registrations and registration amendments.

II. Registrations and other submissions for Committee Review

a. Clinical trial reviews

25-BMC-101, Andrew Mammen

i. Reviewers: Goff, Billioux, All

ii. Review summary: The NIAMS investigators presented a phase 1/2, open-label, dose-escalation and expansion study of CABA-201-002, an autologous CD19-targeted CAR-T cell therapy, in adults and children (≥ 6 years) with refractory idiopathic inflammatory myopathies (IIMs) and juvenile IIM (JIIM) (RESET-Myositis). IIMs (myositis) are a heterogeneous group of rare autoimmune diseases marked by muscle inflammation and weakness; B cell-mediated subtypes include dermatomyositis (DM), anti-synthetase syndrome (ASyS), and immune-mediated necrotizing myopathy (IMNM), while JIIM in patients <18 years also features systemic involvement and, in juvenile DM (JDM), characteristic skin rash. Because no curative therapy exists and standard immunosuppressants carry limited efficacy and serious long-term toxicity (with a subset of patients remaining refractory), CD19 CAR-T-mediated B cell depletion offers a novel approach.

CABA-201-002 (Cabaletta Bio) is manufactured by transducing a patient's T cells *ex vivo* with a third-generation, replication-incompetent lentiviral vector encoding a fully human anti-CD19 scFv (IC78) fused to 4-1BB and CD3ζ signaling domains under an EF1α promoter, using separate gag/pol, VSV-G, Rev, and transfer plasmids to minimize recombination risk. Production will occur at two GMP facilities in Philadelphia. The release testing will confirm the CAR expression, sterility, absence of replication-competent lentivirus, and a vector copy number ≤5 copies/cell. Interim data from 11 adult patients treated in a separate clinical study showed predominantly Grade 1-2 cytokine release syndrome (CRS), no neurotoxicity, peak CAR-T expansion around Day 13, and rapid B cell depletion by one week, along with signs of clinical activity in refractory autoimmune diseases.

The primary objective of the phase 1 study is to evaluate the safety and tolerability of CABA-201 in subjects with active 11M and JIIM over 28 days. The secondary objectives include CAR-T kinetics, manufacturing success, changes in autoantibody titers, and clinical efficacy measured by the Total Improvement Score over 156 weeks. Co-Investigator Dr. Hannah Kim supports pediatric enrollment. Trial activation is planned for 01 October 2025 under Protocol Version 3.0. In Phase 1, up to 24 patients with active myositis (adults and children ≥6 years who have failed ≥3 standard therapies) will be enrolled in three dose cohorts ($3x10^5$, $1x10^6$, and $1x10^7$ cells/kg). Patients will receive fludarabine/cyclophosphamide conditioning on Days -5 to -3. Once a recommended phase 2 dose is identified, an optional expansion of up to 24 additional patients across IIM subtypes will be enrolled. All participants will receive a single IV infusion of CABA-201 on Day 1, will be hospitalized for ≥4 days post-infusion, then followed weekly through Day 28, biweekly through Week 144, and annually through 15 years.

iii. Committee discussion:

- The route of CABA-201 administration needs to be corrected in the registration from 'subcutaneous' to 'intravenous,' and the chain of custody, storage, and delivery processes for the investigational product need to be specified.

- The investigators need to clarify the expected CAR-T and B-cell kinetics in myositis patients, reconciling the reported reconstitution patterns with published lymphoma and autoimmune data.
- DURC response for question on "potential harm" to be amended to "No" to accurately reflect that CABA-201 is an autologous, non-replicative therapy with no dual-use potential.
- The preliminary dose-escalation cohorts have completed the study, and the subsequent participants will uniformly receive the $1x10^6$ cells/kg dose.
- Reevaluate the "failed ≥3 therapies" enrollment criterion, especially for dermatomyositis (patients may only receive corticosteroids), to avoid excluding patients who would benefit but have not exhausted every treatment. The trial will generally enroll patients newly diagnosed with dermatomyositis (on combination corticosteroids and methotrexate). In addition, patients who fail IVIg or JAKi treatment (relapsed-refractory disease) will be eligible for the study. Patients eligible for the study will have active disease despite exposure to ≥3 treatments.
- The investigators to provide a comprehensive list of all active US and international clinical trial sites currently enrolling for CABA-201.

iv. Expected training is BBP for anyone handling clinical samples, and PPE as appropriate for standard precautions and as determined by risk assessment including gloves, gown, and face protection would help mitigate risks of inadvertent exposure during agent preparation and administration, especially when working outside of primary containment such as a Biological Safety Cabinet (see other protocol details in registration).

v. Animal studies proposed: N/A

vi. The committee discussed the dual-use and ePPP potential of these experiments and agreed that there were no dual-use or ePPP concerns with this clinical protocol.

vii. There were no additional public comments.

viii. The clinical protocol is approved with standard precautions as described in place for handling therapeutic agent.

ix. Relevant sections of the NIH Guidelines: III-C-1

x. The committee unanimously approved as written.

xi. There were no conflicts of interest indicated.

25-BMC-102, Hong Ha Rosa Nguyen

i. Reviewers: Billioux, Goff, All

ii. Review summary: The NCI investigators proposed a first-in-human phase 1/2, open-label trial of hCT3-ARTEMIS® T cells, an autologous, glypican-2 (GPC2)-targeted antibody-TCR (abTCR) construct, in children and young adults (ages 3 to 39) with relapsed or refractory high-risk neuroblastoma. Neuroblastoma, arising from neural crest cells, represents the most common extracranial pediatric solid tumor, accounting for 15% of childhood cancer deaths despite intensive multimodal therapy. Only 50% of high-risk patients achieve long-term remission. hCT3-ARTEMIS combines a fully human anti-GPC2 scFv (hCT3) grafted onto a γδ TCR scaffold, which conveys HLA-independent antigen recognition and minimal mispairing with endogenous αβ TCRs, along with a CD30 co-stimulatory domain to amplify T-cell activation at low antigen densities. Preclinical *in vitro* and *in vivo* comparisons demonstrate superior tumor clearance versus conventional CAR-T cells, particularly in models with intermediate-to-low GPC2 expression. The lentiviral transduction uses a 3rd generation packaging system (gag/pol, VSV-G, Rev, and transfer plasmids) to ensure replication incompetence, and manufacturing occurs under GMP at Eureka Therapeutics, with release testing for CAR expression, vector copy number (≤5 copies/cell), sterility, and absence of replication-competent virus. In phase 1, up to

18 participants in a standard 3+3 design will receive lymphodepletion (fludarabine/ cyclophosphamide) followed by escalating doses (e.g., $2x10^6$ to $1x10^7$ cells/kg) of hCT3-ARTEMIS. The primary objective is to define the recommended phase 2 dose and assess safety (dose-limiting toxicities, cytokine release syndrome, neurotoxicity). The subsequent phase 2 expansion (up to 16 patients) will utilize an optimal 2-stage design (null response rate 5%; target 25%) to evaluate overall response (CR/PR/SD) according to International Neuroblastoma Response Criteria. Secondary and exploratory endpoints include T-cell persistence, correlation of tumor GPC2 levels with response, biomarker analyses, and assessment of tumor heterogeneity. Disease evaluations will include ^{123}I-MIBG scan–based Curie scoring, MRI-determined tumor volume changes, and qualitative assessments of bone marrow. Objective responses (CR, PR) will be evaluated according to the International Neuroblastoma Response Criteria, encompassing primary solid-tumor response, metastatic soft-tissue and bone-site responses, and bone marrow metastasis status. Participants undergo inpatient monitoring for at least 4 days post-infusion, with frequent clinical and laboratory assessments through Week 28, biweekly visits through Week 144, and long-term follow-up extending to 15 years. Trial activation is scheduled for 11 May 2026, under protocol version 1.0.

iii. Committee Discussion:

- The investigators will estimate inflammatory toxicity by monitoring for cytokine release syndrome rates compared to CAR-T therapies and report early CRS outcomes once available.
- The statement on lentiviral vector mutagenesis should be corrected by replacing the outdated 1989 reference with current safety data and recent case reports.
- Transduction efficiency statistics from patient-derived T cells (target 70-80% transduction) need to be added to the protocol, and the investigators need to confirm consistency with healthy-donor benchmarks.
- The γδ TCR's mispairing is advantageous over αβ TCRs and is an emerging method. The added CD30 co-stimulatory domain does not signal constitutively but requires GPC2 engagement to amplify T-cell activation. The construct utilizes γδ TCR constant regions, which are similar to those used in αβ TCR engineering, a technique that has been safely employed for over 20 years.

iv. Expected training is BBP for anyone handling clinical samples, and PPE as appropriate for standard precautions and as determined by risk assessment including gloves, gown or labcoat, and eye protection and as described (see in registration).

v. Animal studies proposed: N/A

vi. The committee discussed the dual-use and ePPP potential of these experiments and agreed that there were no dual-use or ePPP concerns with this clinical protocol.

vii. There were no additional public comments.

viii. The clinical protocol is approved with standard precautions as described in place for handling therapeutic agent.

ix. Relevant sections of the NIH Guidelines: III-C-1

x. The committee unanimously approved as written.

xi. There were no conflicts of interest indicated.

b. Special reviews

PRD 7867 amend 250604, Jeffery Taubenberger

i. Reviewers: Craigie, Denny, All

ii. Review summary: The NIAID investigators proposed an amendment to consolidate all murine and ferret vaccine challenge studies for SARS-CoV-2 and related β-coronaviruses under ASP

LID-6E while maintaining culture of wild-type virus at BSL-3 (33/3E14) for neutralization assays. Mouse and ferret studies will evaluate immunogenicity, protective efficacy, and pathogenesis.

iii. Committee Discussion: Studies as described in LID-6E related to analyses from vaccine challenge studies in line with as has been previously approved.

iv. Training in addition to regular requirements to also include ABSL-3 and proficiency training. PPE requirements in-line with regular laboratory requirements for this level.

v. Animal studies proposed: Yes, murine and ferret as discussed in LID-6E.

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposed amendment.

vii. There were no additional public comments.

viii. Work is approved at: Animal work at ABSL-3 practices and containment

ix. Relevant sections of the NIH Guidelines: Not applicable; no recombinant work in this amendment

x. The committee unanimously approved as written.

xi. There were no conflicts of interest indicated.

PRD 8016 amend 250604, Daniel Douek

i. Reviewers: Whitehead, Craigie, All

ii. Review summary: The NIAID investigators proposed an amendment to include sequencing and qRT-PCR analysis of inactivated bronchoalveolar lavage and nasal swab samples from MERS-CoV-infected nonhuman primates, using the same RNAzol BD inactivation protocol previously validated for SARS-CoV-2. No live-virus work, new animal challenges, or additional manipulations are introduced. There is no need to reestablish the inactivation procedure for MERS, as the viruses are similar.

iii. Committee Discussion:

- Blood samples were removed from the scope because inactivation data for blood had not been provided; investigators must validate and demonstrate the efficacy of their MERS-CoV inactivation protocol for blood samples before including them.

iv. Training and PPE requirements as already established for protocol.

v. Animal studies proposed: Yes, nothing new proposed in this amendment.

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.

vii. There were no additional public comments.

viii. Work is approved at: Eukaryotic work at BSL-2; animal work at ABSL-3 practices and containment

ix. Relevant sections of the NIH Guidelines: Not applicable; no new rDNA work proposed with this inactivation procedure.

x. The committee unanimously approved as written.

xi. There were no conflicts of interest indicated.

25-BMC-105, William Figg

i. Reviewers: Craigie, Denny, All

ii. Review summary: The NCI investigators proposed to register temporary receipt, storage, and downstream analysis of clinical pharmacokinetic samples containing West African–clade monkeypox virus that have been inactivated off-site in the Democratic Republic of Congo using a 3:1 methanol-to-plasma ratio (final methanol concentration 10%). Because West African clade monkeypox is not a CDC select agent, these inactivated specimens—plasma, nasal swabs, and other bodily fluids are shipped frozen on dry ice under a CDC PHS 20231103-4093A

import permit and triple-contained per 42 CFR 71.54. Upon arrival, samples will be stored at -80°C in NIH freezers (Room 5A03, Rack 058 O3). No live-virus work or further inactivation is planned; all assays use only the methanol-treated material.

iii. Committee Discussion:
- Formal validation data demonstrating that the 10% methanol protocol reliably inactivates monkeypox in each sample matrix needs to be provided before further handling. Confirm whether additional sample types (e.g., blood) are included and, if so, require corresponding inactivation validation for those matrices. Insufficient data provided and, therefore, it is not convincing that inactivation has occurred.
- Clarify the exact freezer and room location(s) where samples will be stored and ensure inventory tracking is up-to-date.
- Protocol documentation, machine-translated from French, includes annotated handwritten sample lists (positive/negative by index) and shipping manifests, but lacks on-file validation data confirming methanol efficacy.

iv. Training and PPE requirements were undefined, but would be established if approved.

v. Animal studies proposed: No

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the inactivation proposal.

vii. There was no additional public comment.

viii. The work has been TABLED and is not approved.

ix. Relevant sections of the NIH Guidelines: Not applicable; no recombinant work considered.

x. The committee TABLED the submission for additional information including data demonstrating the procedure performs the inactivation as stated.

xi. No conflicts of interest were indicated.

c. Registrations for committee review

25-BMC-103, Brian Brooks

i. Reviewers: Denny, Goff

ii. Review summary: The NEI investigators proposed a pilot study in day gecko lizards (*Phelsuma laticauda*) to probe the limits of retinal regeneration by combining two established neuronal tracers with optic nerve axotomy. Following anesthesia with alfaxalone (30 mg/kg IM) and medetomidine (0.1 mg/kg IM), unilateral intravitreal injection of cholera toxin subunit B-Alexa Fluor 488 (CTB-AF488; 1 µL at 1 µg/µL) will label regenerating retinal ganglion cell (RGC) axons, while a replication-deficient AAV9-CAG:GFP vector (Vector Biolabs; 1 µL at $1x10^{13}$ vg/mL) will drive stable GFP expression in RGCs. AAV9 features AAV2 inverted terminal repeats flanking the GFP transgene, which is under the control of the ubiquitous CAG promoter. Endpoints include *in vivo* visual function tests (electroretinography and optokinetic tracking), longitudinal behavioral assays (prey-capture and obstacle navigation), and high-resolution fluorescence imaging of optic nerve tracts at Days 3, 7, and 14 post-axotomy. Multi-omics analyses on dissected retinas at each time point will consist of bulk and single-cell RNA sequencing, ATAC-seq for chromatin accessibility, and spatial transcriptomics to map regenerative gene expression. The vector map and ASP were provided.

iii. Committee Discussion:
- Suggested streamlining the “Recombinant Material” section title (e.g. “AAV9-CAG:GFP for RGC labeling”) to avoid long narrative sentences that propagate throughout the application.

iv. Training and PPE requirements as established with BSL, to include laboratory safety training.

v. Animal studies proposed: Yes. Gecko lizards

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.
vii. There were no additional public comments.
viii. Work is approved at: Animal work for AAV and tracer injections and containment at ABSL-1; ABSL-2 practices only if shedding or high-titer administered.
ix. Relevant sections of the NIH Guidelines: Animal work at III-D-4-a
x. The committee unanimously approved with minor modifications.
xi. There were no conflicts of interest identified.

25-BMC-104, Cynthia Tifft

i. Reviewers: Billioux, Denny
ii. Review summary: The NHGRI investigators proposed to use bicistronic AAV9-mediated gene replacement therapy for Tay-Sachs (HEXA deficiency) and Sandhoff (HEXB deficiency) diseases using established murine knockout models. GM2 gangliosidosis results from the loss of β-hexosaminidase A (α+β subunits) in $Hexa^{-/-}$ (Tay-Sachs model) and $Hexb^{-/-}$ (Sandhoff model) mice, leading to GM2 accumulation, neurodegeneration, motor deficits, and a shortened lifespan. The study will compare two delivery routes—retro-orbital intravenous injection (neonatal pups: $1x10^{11}$ vg in 10 µL; adults: $1.5x10^{12}$ vg in 150 µL) and intracerebroventricular injection (2 µL at 5×10^{10} vg) of a single AAV9 vector encoding human HEXA and HEXB under a ubiquitous CAG promoter, produced by Dr. McGuinness's laboratory at University of Massachusetts Amherst. Cohorts comprise 20 $Hexa^{-/-}$ and 20 $Hexb^{-/-}$ mice per route, along with 40 wild-type controls. Animals will be monitored weekly for weight, neurologic function (rotarod, grip strength), and survival; brain tissues will be harvested at 4 and 6 months for region-specific transduction analysis (qPCR, immunohistochemistry) and GM2 clearance assays. Endpoints include median survival extension, motor performance improvements, vector biodistribution, and biochemical correction; mice exhibiting >15 % weight loss or severe distress will undergo humane euthanasia. The vector map, ASP, and ASP amendment were provided.
iii. Committee Discussion: No additional discussion after review.
iv. Training and PPE requirements as established with BSL, to include laboratory safety training.
v. Animal studies proposed: Yes. Mice
vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.
vii. There were no additional public comments.
viii. Work is approved at: Animal work at ABSL-1 practices and containment
ix. Relevant sections of the NIH Guidelines: Animal work at III-D-4-a
x. The committee unanimously approved as written.
xi. No conflicts of interest were identified.

25-BMC-106, Sanjay Desai

i. Reviewers: Goff, Whitehead
ii. Review summary: The NIAID investigators proposed to generate and characterize genetically modified *Plasmodium falciparum* lines using a combination of fluorescent/luminescent reporter integration and targeted CRISPR/Cas9-mediated gene edits to probe parasite biology and drug-target interactions. Six laboratory strains (3D7, Dd2, HB3, GB4, 7G8, and Indo) will be cultured in O^+ human erythrocytes at 2-5% hematocrit in RPMI 1640 supplemented with Albumax II. Transfections will employ electroporation of synchronized ring-stage parasites with three plasmids: pUF1-Cas9 (expressing SpCas9 and hDHFR), pL6-eGFP (donor template for eGFP knock-in under the constitutive calmodulin promoter), and pUC57-amp (guide RNA and homology arms), followed by selection with WR99210 and blasticidin for 2-3 weeks. PCR, Southern blot, and fluorescence microscopy will confirm successful integrants. Reporter lines

will be used in real-time growth and invasion assays, while CRISPR knockdowns of candidate drug-target genes (e.g., PfATP4, PfCRT) will be assessed for altered sensitivity to antimalarial compounds in dose-response curves. All human blood is sourced from screened, anonymous donors; waste cultures will be heat-inactivated before disposal. Some of the vector maps, primer sequences, and editing strategies were provided.

iii. Committee Discussion:
- Request a detailed list of all intended genetic modifications (reporter insertions vs. functional gene edits) and their molecular constructs to assess biosafety and functional impact.
- Recommend specifying selection markers, promoters, and guide-RNA targets used in CRISPR/Cas9 to ensure traceability and minimize off-target effects.
- The investigator to clarify whether any edits could confer altered virulence or drug-resistance phenotypes and proposed risk-mitigation strategies, "biological phenotypes" too unclear

iv. Training and PPE requirements as established with BSL, to include laboratory safety training, and BBP training for individuals working with human materials.

v. Animal studies proposed: No

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.

vii. No additional public comments were given.

viii. Work is approved at: Prokaryotic work at BSL-1, eukaryotic work at BSL-2

ix. Relevant sections of the NIH Guidelines: Prokaryotic work at III-D-2-a, eukaryotic work at III-D-3-a

x. The committee unanimously approved this protocol only after additional information is given: (1) What is "the gene product"? Provide vector maps for the DDD post-translational degradation vector, the glmS riboswitch, and the LoxP-introns, (2) What are the epitope tags? What are the reporters? What are the genes to be tagged? Provide a vector map for a protein with its tag or reporter, and (3) What are the mutations, as we need to know what is being mutagenized and how?

xi. No conflicts of interest were identified.

25-BMC-107, Mioara Larion

i. Reviewers: Denny, Craigie

ii. Review summary: The NCI investigators proposed to model IDH1-driven gliomagenesis by generating stable human glioma cell lines and transgenic mice that overexpress oncogenic IDH1 and IDH2 variants. DNA constructs encoding wild-type and tumor-relevant point mutations (e.g., IDH1 R132H, IDH2 R172 K) were designed and cloned by Dr. Kusang Yang; prokaryotic amplification was performed using standard *E. coli* transfection methods. For eukaryotic studies, U-251 glioma cells will be transduced with VSV-G–pseudotyped, 2nd-generation lentiviral vectors (pCMV-IRES-HEXA/HEXB backbones repurposed for IDH genes), and stable integrants will be selected with puromycin. These engineered cells will undergo untargeted metabolomics (LC-MS/MS) and ^{13}C tracing to quantify 2-hydroxyglutarate production and identify accelerated metabolic pathways. Parallel in vivo work involves xenografting IDH1/IDH2 mutant and wild-type U-251 cells into immunocompromised mice via subcutaneous, intracerebral, and oral gavage routes to assess tumor growth kinetics, systemic metabolite profiles (blood, urine, CSF), and survival. Sample collection at defined time points will inform both metabolomic analyses and histopathological evaluations. The vector maps and ASP were provided.

iii. Committee Discussion: None

iv. Training and PPE requirements as established with BSL, to include laboratory safety training.
v. Animal studies proposed: Yes. Mice
vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.
vii. No additional public comments were given.
viii. Work is approved at: Prokaryotic work at BSL-1, eukaryotic work at BSL-2/3, animal work at ABSL-2 practices and containment
ix. Relevant sections of the NIH Guidelines: Prokaryotic work at III-D-2-a, eukaryotic work at III-D-3-a, animal work at III-D-4-a
x. The committee unanimously approved as written.
xi. No conflicts of interest were identified.

25-BMC-108, James Inglese

i. Reviewers: Whitehead, Billioux
ii. Review summary: The NCATS investigators proposed to harness the Bac-to-Bac baculovirus expression system in *Spodoptera frugiperda* (Sf9) insect cells to produce gram-scale quantities of human G-protein-coupled receptor kinase 5 (GRK5) tagged with an N-terminal HiBiT peptide and C-terminal deca-histidine (His_{10}) for use in ultra-high-throughput screening. Codon-optimized cDNAs encoding wild-type GRK5 and the R386S kinase-dead variant were synthesized by Genscript and subcloned into the pFastBac1 vector under control of the polyhedrin promoter. Recombinant bacmids are generated in *E. coli* DH10Bac by Tn7-mediated transposition and selected on gentamicin/ chloramphenicol plates; baculovirus stocks are amplified through two rounds of Sf9 infection at a multiplicity of infection (MOI) of 0.1-0.5. Infected Sf9 cultures will be harvested at 72 hours post-infection; cell pellets will be lysed, and extracted proteins will be verified by SDS-PAGE, Western blot (anti-HiBiT), and NanoBiT luminescence assays. Purified GRK5-HiBiT-His_{10} will be used to study ligand-binding and ADP-generation kinase assays, facilitating the discovery of novel small-molecule inhibitors. The vector maps were provided.
iii. Committee Discussion:
    - The investigators to include a clear, statement of project aims and the biological role of GRK5 and the HiBiT tag to aid in reviewer understanding.
    - Describe the method for verifying baculovirus titers and infection efficiency (e.g., plaque assays or qPCR) in Sf9 cultures.
    - Explain the rationale for choosing N- versus C-terminal HiBiT tagging and provide any preliminary data on tag impact for GRK5 kinase activity.
iv. Training and PPE requirements as established with BSL, to include laboratory safety training.
v. Animal studies proposed: No
vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.
vii. No additional public comments were received.
viii. Work is approved at: Prokaryotic work at BSL-1, eukaryotic work at BSL-2
ix. Relevant sections of the NIH Guidelines: Prokaryotic work at III-D-2-a, eukaryotic work at III-D-3-a
x. The committee unanimously approved with minor modifications.
xi. No conflicts of interest were identified.

d. Registration amendments for committee review

RD-24-II-28 amend 250604, Abdul Banday

i. Reviewers: Goff, Billioux

ii. Review Summary: The NCI investigators proposed an amendment to expand their original CRISPR/Cas9 knockout studies of gene X in human bladder cancer cell lines by adding lentiviral overexpression of upstream regulators and shRNA knockdown of downstream effectors. Newly introduced third-generation, self-inactivating lentiviral vectors carrying candidate regulator cDNAs or shRNA constructs will be used to transduce T24 and UM-UC-3 cells. *In vitro* assays (proliferation, migration, and Matrigel invasion) will be used to assess phenotypic changes, followed by orthotopic and subcutaneous xenograft models in NSG mice to evaluate effects on tumor growth, metastatic potential, and pathway activation (via Western blot and immunohistochemistry). The updated vector maps and genes of interest were provided.

iii. Committee Discussion:

- Highlight newly added overexpression and knockdown experiments with change-tracking markers to distinguish amended content clearly. Provide a concise "Amendment Scope" summary in the registration document, listing added genes, cell lines, and animal assays.
- Detail any gain-of-function risks associated with overexpressed regulators and describe vector safety features (e.g., self-inactivating LTRs).
- Include brief biological rationales for each upstream regulator and downstream effector target to aid safety and relevance assessment.

iv. Training and PPE requirements as already established.

v. Animal studies proposed: Yes. Mice

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.

vii. No additional public comments were received.

viii. Work is approved at: Prokaryotic work at BSL-1, eukaryotic work at BSL-2, animal work at ABSL-2 practices and ABSL-1 containment

ix. Relevant sections of the NIH Guidelines: Prokaryotic work at III-D-2-a, eukaryotic work at III-D-3-a, animal work at III-D-4-a

x. The committee unanimously approved with minor modifications.

xi. No conflicts of interest were identified.

24-BMC-232 amend 250604, Anish Thomas

i. Reviewers: Craigie, Whitehead

ii. Review Summary: The NCI investigators proposed an amendment to their NCI bladder cancer protocol to introduce 2nd generation lentiviral vectors for delivery of CRISPR/Cas9 components targeting both upstream regulators and downstream effectors of a previously studied gene X. New stably transduced human bladder cancer cell lines will be generated in vitro to assess gene editing efficiency and resultant phenotypic changes, then implanted into immunodeficient mice for evaluation of tumor growth, invasion, and molecular pathway alterations. The amendment includes updated vector maps, descriptions of new cell lines, and a revised animal protocol outlining implantation techniques, monitoring schedules, and endpoint analyses.

iii. Committee Discussion:

- The cell lines for the proposed eukaryotic work need to be specified.
- The investigators to provide more details on the proposed work with lentiviruses.

iv. Training and PPE requirements as established.

v. Animal studies proposed: Yes. Mice

vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.

vii. No public comments were given.

viii. Work is approved at: Eukaryotic work at BSL-2/3, animal work at ABSL-1 practices and containment

ix. Relevant sections of the NIH Guidelines: Eukaryotic work at III-D-3-a; animal work at III-D-4-a
x. The committee unanimously approved with minor modifications.
xi. No conflicts of interest were identified.

25-BMC-063 amend 250604, Niki Moutsopoulos

i. Reviewers: Whitehead, Craigie
ii. Review Summary: The NIAID investigators proposed an amendment to their ongoing germ-free mouse colonization study (Registration 25-BMC-063) by introducing a recombinant *Bacteroides ovatus* Δasn strain. Using the pExchange-tdk suicide vector in *E. coli* S17, they will delete the asparagine synthetase gene via homologous recombination and verify mutants by PCR. Germ-free C57BL/6J mice will be mono-associated with either wild-type or Δasn *B. ovatus* through oral gavage, followed by longitudinal fecal sampling for colony counts and targeted metabolomics. Mice will be euthanized 1-4 weeks post-colonization to harvest intestinal tissues for amino acid profiling and characterization of tuft cells. The vector maps and ASP were provided.
iii. Committee Discussion:
- The investigators to provide more details on the proposed prokaryotic work, including details around the construct.
- Map of recombinant vector pGM-NACB-PBT1311-Bovatus_02827 needs to be provided.
- Section K of the ASP to be updated with the registration document number.

iv. PPE and training requirements as established.
v. Animal studies proposed: Yes. Mice
vi. The committee discussed the dual-use and ePPP potential of these experiments. The committee agreed that there were no dual-use or ePPP concerns with the proposal.
vii. No public comments were given.
viii. Work is approved at: Prokaryotic work at BSL-1, animal work at ABSL-2 practices and containment
ix. Relevant sections of the NIH Guidelines: Prokaryotic work at III-D-2-a, animal work at III-D-4-a
x. The committee unanimously approved with minor modifications.
xi. No conflicts of interest were identified.

III. Committee Review of Inactivation Procedures: None other than as reviewed in 8016 amend 250605 and 25-BMC-105.

IV. Standard Operating Procedures/Plans: None

V. Serious Adverse Events in Clinical Trials reviewed by the Committee: None

Reports

I. Biosafety Officer Report: In April 2025, there were 27 research-related incidents. None (apparent) involved work with rDNA, 14 sharps-related incidents, 12 related to work with NHPs, 7 involving research with other animals, 3 related to potential exposures to HBBF, and 1 involved potential exposure to "other" human pathogens.

The BSO report includes research-related incidents in all labs (even in the Clinical Center) but excludes routine patient-care events, which are managed separately. Longstanding practices exist between hospital safety and research biosafety related to documentation and reporting, and efforts are underway to unify incident tracking across both domains.

II. Other reports: None

Around the Room/Committee Discussion

I. As of June 2025, all IBC minutes will be publicly posted in a streamlined, uniform format across the four NIH IBCs; members have reviewed the new template and must recuse themselves from discussions where they have conflicts of interest (as they have always done).

II. A new campus-wide sharps-injury committee is being formed, chaired by the Biosafety Officer, to review research-related sharps incidents quarterly. Committee members were encouraged to volunteer.

Adjournment

The meeting was adjourned by Dr. Malech at 4:01 PM.

Next meeting: July 09, 2025