Institutional Biosafety Committee:

Present: Dr. Steven Albelda, Dr. Julian Baptiste, Dr. Paul Bates, Dr. Jessica Buchanan, Dr. Sara Cherry, Dr. Sherrill Davison, Dr. Joseph Fraietta, Dorothy Kaplan, Dr. Daniel Kessler, Dr. Andrew Maksymowych, Dr. Maureen O'Leary, Dr. David Pegues

Absent:

Invited Guests: Ms. Stephanie Adams, Dr. Sarah Capasso, Ms. Marie-Luise Faber, Ms. Amanda Wong, Ms. Kimberly Craig, Ms. Denene Wambach

The Institutional Biosafety Committee Meeting was called to order by Dr. Daniel Kessler at **10:00 AM**.

1. IBC Minutes: **05-19-2025**

- The IBC reviewed the IBC Minutes.
- All members are in favor of approval as submitted.
- Minutes approved as submitted.

2. Registrations to Review:

SECTION III–C. Experiments Involving Human Gene Transfer that Require IBC & IRB Approval <u>Prior to</u> Initiation:

1. Chapin ....**#25-175** **C–1**

Dr. William J Chapin – NEW HGT Protocol Registration .... **FULL REVIEW**

PROTOCOL TITLE UPCC 06225: Phase II study of neo25-175adjuvant RP2 in combination with preoperative FLOT for patients with stage II or higher, non-metastatic gastroesophageal adenocarcinoma. (Protocol V2 dated May 15,2025; Main ICF dated May 20, 2025).

IBC #25-175, IRB # 858613, Protocol # 0306 IND # 31637

- Dr. Daniel Kessler introduced the submission.
- Dr. Steven Albelda provided a summary and analysis.

"**Sponsor:** Replimune

Penn PIs: Dr. William Chapin and Jennifer Eads

Trial design: This is a single-arm, phase II study evaluating the addition of intra-tumoral injections of RP2 virus to standard of care perioperative FLOT for patients with stage II or higher, non-metastatic esophageal, gastroesophageal junction (GEJ), or gastric adenocarcinoma. The hypothesis is that the addition of RP2 to perioperative FLOT will be safe and will significantly improve pathologic complete response (pCR) rate compared to the historical weighted average of 12% observed with perioperative FLOT in previous trials.

The primary objective is to assess the pCR rate following neoadjuvant RP2 and preoperative FLOT in patients with loco-regional esophageal, GEJ, or gastric adenocarcinoma who undergo surgical resection. Secondary objectives will be safety, disease-free and overall survival.

The study population will include up to 34 adult patients with newly diagnosed clinical T2 or higher or any node positive, non-metastatic esophageal, GEJ, or gastric adenocarcinoma that are eligible for perioperative FLOT followed by surgical resection.

Following enrollment, the study intervention will include upper endoscopy with intra-tumoral injection of $1x10^6$ plaque forming units per mL (PFU/mL) of RP2 within 4 days prior to the first cycle of FLOT (5-FU, leucovorin, oxaliplatin, docetaxel). Intra-tumoral injection of $1x10^7$ PFU/mL of RP2 will occur within 4 days prior to of the following three cycles of pre-operative FLOT. FLOT will be administered starting day 1 of each 14 day cycle for 4 cycles. Patients will subsequently undergo surgical resection 4-6 weeks following the completion of the preoperative element of FLOT. Patients will be planned to undergo standard of care management post-operatively with 4 additional cycles of FLOT.

This study will be monitored in accordance with the Cancer Center's Clinical Trials Scientific Review and Monitoring Committee (CTSRMC) Plan, approved by National Cancer Institute (NCI) during the Core Grant's most recent review.

Biosafety Data: RP2 is a selectively replication competent, acyclovir-sensitive HSV-1 virus. RP2 is the second in a series of viruses (RP1, RP2, and RP3) being developed by Replimune, with each virus modified as compared to its predecessor by the insertion (or deletion) of additional transgenes.

RP2 was constructed using a new strain of HSV-1 (strain RH018). The neurovirulence factor (infected cell protein [ICP] 34.5) encoding genes and the ICP47-encoding gene are deleted from the virus making replication tumor-selective. The virus also contains a codon-optimized sequence for human granulocyte-macrophage colony-stimulating factor (hGM-CSF) in addition to a codon-optimized sequence for the gibbon ape leukemia virus surface protein (GALV-GP) with the R sequence deleted (R-). Cell-to-cell fusion is caused by GALV-GP-R-, resulting in accelerated cell death. Further, RP2 expresses a human cytotoxic T lymphocyte antigen 4 blocking antibody-like molecule (ahCTLA-4), which interferes with the interaction of cytotoxic T lymphocyte antigen 4 (CTLA-4) with B7 molecules on professional antigen presenting cells.

A first generation HSV1 virus (lacking the neurovirulence genes ICP 34.5 and ICP47, and expressing GM-CSF) was FDA approved in 2015 for IT injection in melanoma and has proven safe.

Antitumor efficacy of a mouse version of RP2 has been demonstrated in immune-competent mice with HSV-1-permissive mouse tumors (A20) and with RP2 in hCTLA-4 knock-in immune-competent mice with MC38 mouse tumors. Consistently, tumor regression or clearance was seen following intratumoral administration of RP2 at a range of virus doses in both injected and noninjected tumors. No serious toxicity was seen.

A number of patients have been treated with RP2 in a clinical trial (RP2-001-18) that included patients with advanced or metastatic solid tumors (including melanomas, sarcomas, pancreas tumors, colorectal, and head and neck cancers). As of the cutoff date of 20 December 2024, 85 patients had been treated in Study RP2-001-18. 25 patients with RP2 monotherapy and 60 patients with RP2 and nivolumab. Of the 60 patients enrolled in a cohort that received RP2 in combination with nivolumab, all received at least 1 dose of RP2, and 50 patients have received at least 1 dose of nivolumab.

Dose escalation (Part 1) of the phase 1 is complete (RP2-001-18). Doses of RP2 from 1×10^5 to 1×10^7 PFU/mL were studied without reaching a maximum tolerated dose in either of the dose escalation cohorts. The study safety review committee determined the recommended phase II dose of RP2 to be a single dose of 1×10^6 PFU/mL, followed by up to 7 doses of 1×10^7 PFU/mL.

Safety results showed that RP2 was well tolerated. Nine of 85 (10.6%) patients had treatment-emergent adverse events (TEAEs) that led to discontinuation of study medication. Commonly reported RP2-related treatment-emergent adverse events (TEAEs) include pyrexia, chills, fatigue, influenza-like illness, and injection-site reactions. Overall, 19 patients had a TEAE resulting in death; none of the deaths were considered related to RP2. Overall, 9 patients discontinued RP2 due to a TEAE. A preliminary efficacy analysis of all subgroups with the cutoff date of 20 December 2024 was conducted for patients who received RP2 monotherapy or RP2 combined with nivolumab. For patients treated with RP2 monotherapy (n=20), 1 patient had a complete response (CR), and 2 patients had a partial response (PR). For patients who received RP2 combined with nivolumab (n=56), the objective response rate (ORR) was 17.9%.

RP2 DNA was not detected in blood samples at the 30-day, 60-day, or 100-day follow-up visits, demonstrating complete clearance of RP2 DNA from the systemic circulation. The incidence of detection of RP2 DNA was highest in the injection-site swab samples, with RP2 DNA detected in approximately 5-10% of samples for up to 15 days after the injection.

Recommendation: Given the previous clinical experience with this agent, the sensitivity of the RP2 to acyclovir, the protective procedures put in place, and the unmet medical need, I recommend the protocol be approved."

- The new HGT registration was discussed by the committee members.
- All members were in favor of approval.
- The new HGT registration is approved as submitted.

2. Cohen............**#25-150**................ **C-1**

Dr. Adam D Cohen – NEW HGT Protocol Registration .... **FULL REVIEW**

PROTOCOL TITLE: UPCC 41419 A Phase 2, Multicohort Open-Label Study of JNJ-68284528, a Chimeric Antigen Receptor T cell (CAR-T) Therapy Directed Against BCMA in Subjects with Multiple Myeloma. CARTITUDE-2. (Protocol V7 dated December 19, 2024; Main ICF dated January 16, 2025).

IBC #25-150, IRB #834074, IND #18080, UPCC #41419, Protocol #68284528MMY2003

- Dr. Daniel Kessler introduced the submission.
- Dr. Paul Bates provided a summary and analysis.

"Ciltacabtagene autoleucel (cilta-cel) is an autologous chimeric antigen receptor T cell (CAR-T) therapy that

targets B cell maturation antigen (BCMA), a molecule expressed on the surface of mature B-lymphocytes and malignant plasma cells. The cilta-cel drug product used in this study and the LCAR-B38M CAR-T cell drug product used in the first-in-human Legend-2 study express an identical CAR protein. The cilta-cel drug product will be produced using modified manufacturing and scale-up processes. Results from the Phase 1b portion of Study 68284528MMY2001 and the Legend-2 study indicate that cilta-cel and LCAR-B38M CAR-T cells have significant anti-myeloma activity and a safety profile consistent with the known mechanism of action of the product.

The objective of Study 68284528MMY2003 is to determine the safety and efficacy of cilta-cel in various MM treatment settings. Multiple cohorts will run in parallel for enrollment of unique patient populations of unmet medical need. Details of each cohorts A though F are provided in the Overview of Study Design section 3.1.

Agent Description: JNJ-68284528 Ciltacabtagene autoleucel (cilta-cel)

Active Ingredient: Lentivirus modified autologous T cells

Pharmacological Class: CAR T-cell

Viral Construct: Lentivirus

Active Ingredient: Recombinant lentivirus expressing BCMA targeting chimeric antigen receptor (CAR T) using two VHH BCMA-targeting domains

Results from a previous study of cilta-cel in heavily pre-treated patients indicate an ORR of 87.8% with a CR rate of 64.9%. The observed response rates and the reversible adverse events for most subjects, support further investigation of this approach in the current study.

- Is a novel vector system, approach or technology used for this clinical trial? NO
- Gene transfer agent delivery method – ex vivo transduction of autologous T cells which are then infused IV
- Intended target, cells (*ex vivo* or *in vivo*) – T cells ex vivo
- Any other material to be used in preparation of the agent (vector and transgene) to be administered to research participants - NONE
- Biosafety Concerns applicable to clinical staff, subjects, subject families, community - NONE beyond known CRS & ICANS issues.
- Are **"Standard Precautions,"** Biosafety Level 2 (BSL-2) equivalent, practices appropriate for ensuring clinical trials personnel safety? YES

This Registration **Meets IBC Criteria for Approval** - I recommend approval, as submitted."

- The new HGT registration was discussed by the committee members.
- All members were in favor of approval.
- The new HGT registration is approved as submitted.

3. Irwin ....**#24-113** **C–1**

Dr. David Irwin – HGT Protocol Registration Amendment V10 .... **AMENDMENT**

PROTOCOL TITLE: A Phase 1/2 Ascending Dose Study to Evaluate the Safety and Effects on Progranulin Levels of PR006A LY3884963 in Patients with Fronto-Temporal Dementia with Progranulin Mutations (FTD-GRN) (PROCLAIM). (Protocol V10 dated October 11, 2024; Main ICF V8 date January 3, 2025; Travel ICF V3 dated June 1, 2024; SP ICF V5 dated January 3, 2025).

IBC #24-113, IRB #844811, IND #19511, Protocol #J4B-MC-OKAA

- Dr. Daniel Kessler introduced the submission.
- Dr. Jessica Buchanan provided a summary and analysis.

"**Project Overview:** Targeted Disease and/or Clinical Aim of Project: Fronto-temporal dementia with mutaions in the progranulin gene (GRN).

Agent Description: LY3884963 (PR006A) is an investigational rAAV9 vector-based gene therapy. It contains a GRN expression cassette containing a cytomegalovirus enhancer (CMVe) immediate early enhancer and CBA promoter with a FRN cDNA engineered for optimized human codon usage packaged into an AAV9 serotype vector.

The LY3884963 vector insert contains the Chicken Beta-actin (CBA) promoter element, consisting of 4 parts: the CMVe, CBA promotor, Exon 1, and intron to constitutively express the codon-optimized coding sequence of human GRN. The 3' region also contains a woodchuck hepatitis virus posttransciptional regulatory element followed by a bovine growth hormone polyA tail.

Gene insert: GRN

Control elements: chicken beta-actin promoter with CMVe elements

Is a novel vector system, approach or technology used for this clinical trial? NO

Gene transfer agent delivery method: Suboccipital injection into the cisterna magna.

Intended target, cells (ex vivo or in vivo), and transduction efficiency (if available): Targets neurons and other brain cells. Shown in vitro and in vivo to effectively transduce in a dose-dependent manner.

Any other material to be used in preparation of the agent (vector and transgene) to be administered to research participants (e.g., helper virus, packaging cell line, carrier particles): Manufacturing and formulation: the first version of LY3884963 product (PR006A v1.0) is manufactured in a HEK293 system; PR006 v2.0 is manufactured in an Sf9/baculovirus system. LY3884963 finished product consists of rAAV9.CBA.GRN virus vectors presented in aqueous solution composed of 20 mM Tris pH 8.0, 1 mM MgCl 2, and 200 mM NaCl containing 0.001% (w/v) poloxamer 188. It is supplied for clinical use as a sterile frozen solution in single-use vials with 1.0 mL extractable volume and stored at ≤ -60℃.

Potential for shedding: Shedding risk similar to other AAV. NHP studies indicate viral particles below the limit of detection by Day 30. Exploratory Objective will mesure shedding on Day 14, and at 1, 2, 3, and 6 months.

The amendment includes updates to the investigator brochure (IB v11.0) that reflect safety information relating to a fatal SAE of DVT/PE that was experienced by a patient. In total, four patients have experienced DVT/PE events and, although the sponsor has deemed all four to be unrelated to LY3884963, the protocol now includes additional safety monitoring in response to this SAE, including d-dimer testing and Wells Score assessments at all visits, and the exclusion of patients at high risk of DVT due to a hypercoagulable state at screening.

Are "Standard Precautions," Biosafety Level 2 (BSL-2) equivalent, practices appropriate for ensuring clinical trials personnel safety? YES

This Registration Meets IBC Criteria for Approval: YES, I recommend approval, as submitted."

- The amendment was discussed by the committee members.
- All members were in favor of approval.
- The HGT registration amendment is approved as submitted.

4. Irwin..............**#24-135**................ **C–1**

Dr. David Irwin – HGT Protocol Registration Amendment V7'F"... **AMENDMENT**

PROTOCOL TITLE: A Phase 1B Open-Label, Multicenter, Dose Escalation Study to Assess the Safety, Tolerability, and Pharmacodynamic Effects of a Single Dose of PBFT02 Delivered into the Cisterna Magna (ICM) of Adult Subjects with Frontotemporal Dementia (FTD) and Mutations in the Progranulin (GRN) Gene. (Protocol V7, Amendment F dated August 12,2024; Main ICF V7 dated December 12, 2024; SP ICF V7 dated December 12, 2024; Data ICF V1 dated January 8, 2021).

IBC #24-135, IRB #848900, IND #26814, Protocol #PBFT02-001

- Dr. Daniel Kessler introduced the submission.
- Dr. Joseph Fraietta provided a summary and analysis.

"This is an amendment to Protocol PBFT02-001, a Phase 1b (open-label, dose-escalation) study of adeno-associated virus serotype 1 vector AAV1.CB7.CI.hPGRN.rBG delivered via intracerebroventricular (ICV) injection in participants with frontotemporal dementia due to progranulin (FTD-GRN) haploinsufficiency, now extended to include frontotemporal dementia due to C9orf72 hexanucleotide repeat expansions (FTD-C9orf72). So, again, C9orf72 hexanucleotide repeat expansions are a common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), referred to as FTD-C9orf72. These expansions involve a repeating sequence of six nucleotides (GGGGCC) within the C9orf72 gene.

There are several proposed amendments to this study:

1. **Cohort Expansion**
   The protocol adds two new cohorts (Cohorts 4 & 5) enrolling up to 10 participants with confirmed C9orf72 hexanucleotide repeat expansions (>30 repeats), with parallel objectives, endpoints, dose-staggering rules, and safety measures mirroring the original FTD-GRN cohorts.
2. **Schedule of Events Revisions**
   - Serum human chorionic gonadotropin (HCG) testing now applies only to women of childbearing potential.
   - Baseline vector-shedding samples added 1–2 days pre-dose.
   - Magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), and magnetic resonance venography (MRV) shifted into screening (by Day –7).
   - Lumbar puncture and MRI imaging extended through Month 3 (with optional follow-up based on emerging data).

- Remote visits restricted to Principal Investigator (PI)/Sponsor agreement.
- Participant experience survey added at Month 12 to capture qualitative feedback.

3. **Eligibility Criteria Refinement**
   - Inclusion expanded to FTD-C9orf72 (confirmed by Clinical Laboratory Improvement Amendments [CLIA]–certified laboratories).
   - Plasma progranulin (PGRN) screening requirement removed.
   - Exclusion criteria updated (vaccination windows, respiratory support, bilirubin thresholds).
   - COVID-19–specific provisions deleted to reflect current clinical practice.
4. **Safety, Procedural, and Biomarker Enhancements**
   - ICV administration clarified to permit needle repositioning without aborting the procedure.
   - Common Terminology Criteria for Adverse Events version 5 (CTCAE v5) definitions refined for laboratory abnormalities.
   - Vector-shedding rules require three consecutive negative samples before cessation.
   - Post-dose monitoring windows for vital signs and neurological examinations standardized.
   - Immunogenicity endpoints broadened to include:
     - Cellular responses by enzyme-linked immune absorbent spot (ELISpot) assay and intracellular cytokine staining (ICS).
     - Humoral responses via anti-AAV1 and anti-PGRN antibody assays.
   - Secondary and exploratory biomarkers expanded to: plasma/cerebrospinal fluid (CSF) PGRN, neurofilament light chain (NfL), MRI volumetrics, optical coherence tomography (OCT), comprehensive neurocognitive testing, and the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) for C9orf72 participants.

Because there are no changes to the AAV1.CB7.CI.hPGRN.rBG gene-therapy product itself, only to study procedures, schedule, eligibility, and assessments, this Reviewer recommends approval of the proposed amendments."

- The amendment was discussed by the committee members.
- All members were in favor of approval.
- The HGT registration amendment is approved as submitted.

5. Kim................**#25-184** **C–1**

Dr. Benjamin Kim – NEW HGT Protocol Registration .... **FULL REVIEW**

PROTOCOL TITLE: A Randomized, Partially Masked, Controlled, Phase 3 Clinical Study to Evaluate the Efficacy and Safety of RGX-314 Gene Therapy in Participants with nAMD. (Protocol V6.1.1 dated March 10, 2025; Main ICF dated May 29, 2025).

IBC #25-184, IRB #851614, IND #17280, PROTOCOL #RGX-314-3101

- Dr. Daniel Kessler introduced the submission.
- Dr. Maureen O'Leary provided a summary and analysis.

"RGX-314 is a recombinant adeno-associated viral vector, AAV serotype 8, containing a transgene that encodes for soluble anti-vascular endothelial growth factor (VEGF) antigen-binding fragment (Fab) protein. The expressed transgene product exhibits anti-VEGF activity similar to the marketed product ranibizumab, which has established anti-VEGF activity resulting in clinical efficacy and safety in the treatment of nAMD for over 10 years.

Approximately 465 participants aged ≥ 50 and ≤ 89 years with nAMD who meet the inclusion/exclusion criteria will be part of this study.

One clinical study of RGX 314 was completed and another 8 clinical studies of RGX 314 are ongoing. Overall, results from the completed first in human administration study RGX 314 provided initial evidence of the possible benefit of RGX-314 in subjects with nAMD based on generally maintained or improved Best corrected visual acuity and central retinal thickness in all cohorts relative to Baseline at doses ranging from 3 × 109 to 2.5 × 1011

As a background for this study: AMD is a progressive degenerative macular disease, causing pathological changes in the region of highest visual acuity, the macula.

Preventive therapies have demonstrated little effect and therapeutic strategies have focused primarily on treating the neovascular lesions. Excessive VEGF plays a key part in promoting neovascularization and edema in nAMD. Counteracting the effects of VEGF provides significant therapeutic benefit to patients suffering from this disorder.

Available anti-VEGF agents injected intravitreally and approved for nAMD treatment include 5 treatments (pegaptanib sodium, ranibizumab, aflibercept, brolucizumab, and faricimab.) Although the majority of these agents have markedly improved disease management, their effects may be limited in duration of effectiveness.

While long term anti-VEGF therapies may improve vision or slow the progression of vision loss, none of these existing treatments prevent neovascularization from recurring.

Therapy typically must be readministered to maintain its therapeutic effect, as evidenced in the registration trials for ranibizumab (monthly injections) and aflibercept (injections every 2 months after 3 monthly loading doses).

The need for repeat treatments can incur additional risks and is burdensome for patients, caregivers, and the healthcare system.

Thus, there is significant need for a long acting therapeutic. An effective gene therapy product with a sustained duration of action may have a significant benefit for patients by substantially reducing the number of injections required to maintain a positive treatment effect, providing the potential for a profound impact on the treatment of this disease.

As with all therapeutic proteins as well as AAV gene therapies, there is a potential for an immune response. If a subject were to experience an immune response,

Following RGX 314 administration in subjects with nAMD, the majority were acute and limited to the anterior segment of the eye, mild or moderate in severity, which is expected after a vitrectomy procedure, and typically resolved within 14 days.

Since RGX has already shown promise for the treatment of neovascular-age -related Macular degeneration with relatively low risk for side effects, I recommend approval."

- The new HGT registration was discussed by the committee members.
- All members were in favor of approval.
- The new HGT registration is approved as submitted.

6. O'Hara............**#24-011** **C-1**

Dr. Mark H O'Hara – HGT Protocol Registration Amendment V6... **AMENDMENT**

PROTOCOL TITLE: UPCC 25223: A Phase I/II Open-Label Study to Evaluate the Safety, Cellular Kinetics and Efficacy of AZD5851, a Chimeric Antigen ReceptorT-Cell (CAR-T) Therapy Directed Against GPC3 in Adult Participants With Advanced/Recurrent HEpatocellular CarciNomA: ATHENA. (Protocol V6 dated January 14, 2025; Main ICF dated February 6, 2025).

IBC #24-011, IRB #855201, IND #29608, Protocol #AZD5851

- Dr. Daniel Kessler introduced the submission, and provided a summary and analysis.

"**Sponsor:** Replimune **Project Overview:** Targeted Disease and/or Clinical Aim of Project Advanced/recurrent hepatocellular carcinoma (HCC) where first-line standard treatment has either failed or unable to receive CPI treatment. Glypican-3 (GPC3) is present on HCC tumor cells and virtually absent in normal adult tissues, making it an ideal target for CAR T-cell therapy. Early clinical trials of CAR-T cells targeting GPC3 in HCC appear to demonstrate similar safety profiles and have reported antitumor activity.

Agent Description: AZD5851Active Ingredient: autologous chimeric antigen receptor (CAR) T-cell product targeting glypican-3 (GPC3) and armored by a dominant-negative transforming growth factor-beta receptor II (dnTGFβRII) Pharmacological Class: Modified autologous T-cellViral Construct: Lentiviral transduction of T-cells to introduce chimeric antigen receptor

Is a novel vector system, approach or technology used for this clinical trial? NO

Gene transfer agent delivery method: Intravenous infusion of CAR-T product

Intended target, cells (*ex vivo* or *in vivo*), and transduction efficiency (if available): Hepatocellular carcinoma cells

Any other material to be used in preparation of the agent (vector and transgene) to be administered to research participants (e.g., helper virus, packaging cell line, carrier particles): Manufacturing and Formulation: AZD5851 is manufactured using standard CAR-T methods. CD4 and CD8 T-lymphocytes are collected from subject leukapheresis material. Following isolation and activation cell culture is initiated and cells are transduced with LVV to express the GPC3 CAR and dnTGFβRII transgene, and expanded for 4 days. The cells are then washed and concentrated to make AZD5851, which is a cryopreserved liquid cell suspension (10 × 106 viable cells/mL) intended for intravenous infusion.

Are "Standard Precautions," Biosafety Level 2 (BSL-2) equivalent, practices appropriate for ensuring clinical trials personnel safety? YES

This Registration Meets IBC Criteria for Approval: YES I recommend approval, as submitted."

- The amendment was discussed by the committee members.
- All members were in favor of approval.
- The HGT registration amendment is approved as submitted.

HGT Registrations Administrative Actions: **# 12**

Research Registration Administrative Actions: **# 52**

SECTION III-B. NIH/OBA and IBC Approval Before Initiation:

7. Luo................25-148...........**B-1, D-4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of adeno-associated viral vector generated by Penn Vector Core or purchased from vendors to manipulate neurons of interest for expression of proteins involved in signaling pathways, such as channels and receptors, to activate or inhibit neuronal activities. The AAV vectors are administered to mice by stereotaxic injection to the sites of interest, and the consequences for the gene manipulation is examined by monitoring animal behavior post administration. The AAV vector is also used to deliver genes for the catalytic domain of toxin molecules, Diphtheria toxin segment A and Tetanus toxin light chain. The expression of these toxin molecules enables selective ablation of neuronal cells of interest which allow examination of the functions of different types of primary sensory afferents and spinal cord neurons in chronic pain and itch conditions. Containment has been set to BSL-2 for handling AAV vectors carrying the gene for toxin molecules, BSL-1 for handling AAV vectors carrying other genes, ABSL-2 for the administration of AAV vectors carrying the gene for toxin molecules, and ABSL-1 for the administration of AAV vectors carrying other genes. This work was previously approved by the NIH OSP.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

SECTION III–D. Experiments that Require IBC Approval Before Initiation:

8. Carroll............25-014............**D-1, 3**

- This registration was presented by Dr. Sarah Capasso. This registration is for the generation and/or use of a retroviral vector with an amphotropic envelope to introduce a reporter that leads to an increase in fluorescence when cell autophagy increases. The lab will follow the autophagic response of a mutant TP53 gene, a tumor suppressor gene, in acute myeloid leukemia (AML) cell lines after various forms of DNA damage. Cell death will be measured. This will allow the lab to study gene mutations with respect to chemotherapy resistance. The vector will be purchased from Addgene. Containment has been set to BSL-2.
- The registration was discussed by committee members.

- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

9. Cullen............22-055............**D-4**

- This registration was prepared by Dr. Tucker Piergallini and presented by Ms. Marie-Luise Faber. This registration is for the downgraded generation and/or use of adeno-associated viral vector mediated transduction to express fluorescent/luminescent reporter genes, light-inducible ion channels, or neurotrophic factors in these studies. The lab is requesting a downgrade to BSL1 and ABSL1 for human iPSC-derived neurons and muscle cells transplanted into rats or pigs. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members. Dr. Daniel Kessler asked if this follows the new policy. Ms. Maire-Luise Faber and Dr. Sarah Capasso confirmed it does.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

10. Hambardzumya……….25-140………. **D-4**

- This registration was presented by Dr. Sarah Capasso. This registration is for the generation and/or use of an ecotropic retroviral vector, Replication-Competent Avian Sarcoma-leukosis Virus (RCAS) which is a modified avian retrovirus. Chicken embryo fibroblast cells DF-1 will be used to propagate the vector that will express oncogenes known to drive brain tumors. Mouse that are expressing tva, the RCAS virus receptor, will be administered the vector. Brain tumors will form, and this will allow the lab to create and study a mouse model of glioblastoma (GBM). RCAS and TVA expressing mice were gifted from collaborators. Nestin-positive cells in the mouse brain, they can transform these cells into neoplastic cells to create GBM. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

11. Haney............24-217...........**D-1, 3, 4 O-1**

- This registration was prepared by Dr. Tucker Piergallini and presented by Ms. Marie-Luise Faber. This registration is for the generation and/or use of 3rd generation lentiviral vectors. The lab studies imbalances between immune cell types in autoimmune disease, cancer, and other pathologies. They aim to understand the immune landscape for creation of immunotherapies. They will do a genetic screen in mice to discover genes that play a role in immune cell organ homing. They will use a cellular barcoding CRISPR mediated gene editing approach that is tractable in the mammalian immune system using ~200,000 sgRNAs such that each cell contains 1 sgRNA and the pool of cells contains every possible KO cell in the mouse genome. In this approach the cellular phenotypes of perturbations are tracked by deep sequencing so the role of all genes in the genome for a particular phenotype can be tested in one experiment. They will package lentivirus in the lab and transduce bone marrow hematopoietic stem cell (HSCs) isolated from Cas9 expressing mice. HSCs will be

expanded and then transplanted into mice for study. Containment has been set to BSL-2 and ABSL-2.

- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

12. Haney............24-219...........**D-4 O-**1

- This registration was prepared by Dr. Tucker Piergallini and presented by Ms. Marie-Luise Faber. This registration is for the generation and/or use of recombinant Cas9 protein and sgRNA. The lab aims to investigate the molecular mechanisms by which immune cells alter neurodegenerative disease risk, such as Alzheimer's disease. Apoe is one gene that regulates immune cell homing and function. To investigate this gene, they intend to edit primary murine hematopoietic stem cells (HSCs) isolated from C57BL/6 mice using CRISPR-Cas9 technology delivered as ribonucleoprotein (RNP) complexes. The RNPs, composed of recombinant Cas9 protein and chemically synthesized sgRNAs, will be introduced via nucleofection. After transient exposure to the RNPs, edited HSCs will be expanded ex vivo and transplanted into recipient mice. Following hematopoietic reconstitution, they will assess immune cell trafficking and functional phenotypes in both peripheral tissues and the brain. They will focus on Apoe and related Alzheimer's disease genes. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

13. Haney............25-177............**D-4**

- This registration was presented by Ms. Amanda Wong. This registration is for the generation and/or use of adeno- associated viral vectors in mice. The lab is studying neurodevelopment in synaptic engulfment. Using AAV the lab aims to label synapses with a pH-sensitive fluorescent protein. This approach allows selective visualization of synaptic material within the acidic environment of microglial phagosomes, enabling quantitative analysis of synapse engulfment under various conditions. Recombinant AAV will be administered systemically via retro-orbital injection to achieve widespread CNS (central nervous system) transduction. After sufficient expression time, mice will be sacrificed and microglia isolated from brain tissue. Engulfed synaptic material will be assessed using flow cytometry and imaging. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

14. Koumenis.......25-149............**D-4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of pancreatic cancer cell line from primary tumors derived from KPCY mice, which was purchased from Kerafast. The lab is interested in expanding their knowledge of radiotherapy in a clinically setting by monitoring pancreatic ductal adenocarcinomas (PDA) in mice. For this reason, they will employ tumor cells from the KPCY mouse model, which recapitulates major features of the PDA, including mutated Kras and p53, with the addition of a Yellow Fluorescent Protein reporter for monitoring Tumor progression. The tumor cells will be injected into the pancreas. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

15. Koumenis.......25-153............**D-1, 4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. The registration is for the generation and/or use of 4th generation lentiviral vectors from SignaGen Laboratories to modify murine tumor cells (MOC2 and SCC7) expressing TdTomato or Luciferase. The lab will inject the modified cells in mice submucosally (into the anterior tongue) to study head and neck toxicity from irradiation with and without tumors. Containment has been set to BSL-2 and ABSL-2.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

16. Mangalmurti ..25-061............**D-4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This is a registration for the generation and/or use of *Plasmodium berghei* ANKA modified to express mCherry and Luciferase in vivo. The modified *P. berghei* was acquired from the John lab at CHOP. Toll-like receptors (TLR9) receptors emerge on the membrane surface of human red blood cells (RBCs) upon exposure to pathogens (such as viruses, bacteria, or parasites) in the bloodstream, which activates the innate immune response, leading to excessive release of pro-inflammatory cytokines that can aggravate a disease. The lab is interested in the relationship between the expression of TLR9 on RBCs and its impact on disease severity in malaria. They will replicate the physiological conditions during malaria infection using mouse models with mutant rodent malaria parasite P. berghei ANKA (line 1868, mCherry@hsp70-Luc@eef1a). They will track the parasite growth by utilizing mcherry expressing P. berghei strain while simultaneously measuring TLR9 expression via FITC-tagged TLR9 antibodies. Plasmodium-infected RBCs (Red Blood Cells) will be injected into mice through the intraperitoneal route. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

17. Ming..............25-134...........**D-4 O-1**

AND

18. Song..............25-135...........**D-4 O-1**

- The registrations were presented by Ms. Stephanie Adams-Tzivelekidis and are for both the Song and Ming labs. The registrations are for the generation and/or use of plasmids for CRISPR modified murine and human cell lines administered to mice. The labs aim to investigate molecular and cellular mechanisms of neural development and function. They are using the lentiviral vectors to label neural stem cells. Additionally, they will use the CRISPR plasmids expressing CAS9 and guide RNAs in vitro in human and mouse cells to modify genes of interest. CRISPR will be used to modify gene function via insertion or deletion of base pairs and to knock-out gene function via generation of indels. Containment has been set to BSL-2, ABSL-1 for the use of modified murine cell lines and direct injection of plasmids, and ABSL-2 for the use of modified human cell lines.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registrations were approved.

19. Phillips-Cremins...................25-123…………**D-1, 3 O-1**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of 2nd generation lentiviral vectors and CRSIPR in vitro. Vectors were purchased from Addgene. The lab is investigating Fragile X syndrome (FXS), which is a human genetic disease associated with chromosome X. The full mutation usually causes FMR1 silencing. To better understand the genome folding related mechanism that mediated FMR1 silencing, they propose to use dCas9 that is expressed by lentiviral vector to disrupt CTCF binding. Downstream analysis will include RT-qPCR, CUT&RUN, HiC, the results will make a connection between 3D genome folding and FMR1 inactivation and regulation; and provide insight for therapeutical drug design and clinical therapy application in the future. Containment has been set to BSL-2.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

20. Powell...........25-057...........**D-4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of mRNA/DNA LNPs. The lab focuses on developing and improving strategies for T cells-mediated immunotherapy of cancer. Gene engineered T cells are the new generation therapeutic designed to treat cancer and are testing multiple genes to determine their impact on cancer cures. The lab's goal is to develop LNP mediated gene transfer to eliminate viral components by using LNP and explore in vivo gene transfer so that eventually patients can get tailored treatment without the delay of collecting immune cells and transforming them in vitro then re-administering them for immunotherapy. Containment has been set to BSL-2, ABSL-1 for mRNA-LNPs directly injected, or for modified murine cell lines administered into mouse, and ABSL-2 for modified human cell lines administered into mouse

- The registration was discussed by committee members. Dr. Daniel Kessler asked for a more descriptive title.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved pending update to project title.

21. Sasaki............25-136...........**D-1, 3, 4**

- This registration was presented by Dr. Sarah Capasso. This registration is for the generation and/or use of a 3rd lentiviral vector to overexpress human Glial cell line-derived neurotrophic factor (GDNF) and/or Fibroblast growth factor 2 (FGF2) in mouse gonad somatic cells. These two genes are growth factors that play crucial roles in the maintenance of spermatogonial stem cells (SSCs) and spermatogenesis within the testes. Modified cells will be transplanted into mice. This will allow the lab to study failures in germline development that drive male infertility. Containment has been set to BSL-2 and ABSL-2.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

22. Weber............25-162...........**D-1, 4**

- This registration was prepared by Dr. Tucker Piergallini and presented by Ms. Marie-Luise Faber. This registration is for the generation and/or use of Pseudotyped mutant rabies virus. The lab studies sleep in mammals, and its associated abnormalities and their link to psychiatric disorders. The goal of the research is to uncover the neural circuits underlying REM sleep and to understand the mechanisms by which these become dysfunctional in psychiatric disorders. Rabies virus is a powerful transneuronal tracer to explore the neuronal circuits synaptically connected to each other in vivo because it has a property of retrograde transduction via synaptic contacts. The lab will use a virus with a deletion of the B19-glycoprotein, resulting in the virus unable to spread to nearby cells unless they express this gene. The second modification is to alter the tropism of the virus (pseudotyped with avian EnvA) so that it cannot infect any mammalian cells except a genetically specified neuronal population expressing a transgene encoding the receptor (TVA) for EnvA. Thus, the resultant virus is "mono-synaptic" trans-neuronal tracer and has no potential to infect or trans-synaptically transport to any other mammalian cells including humans and mice (who do not express TVA anywhere in the body). Given these modifications, viral shedding from injected rodents is extremely unlikely, because the mutant rabies virus is not capable of propagating in the mouse or rat cells except a genetically specified small subset of cells in the brain expressing the B19 glycoprotein and TVA receptor as transgenes. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

23. Weber............25-163...........**D-4**

- This registration was prepared by Dr. Tucker Piergallini and presented by Ms. Marie-Luise Faber. This registration is for the generation and/or use of adeno-associated viral vectors. The lab studies sleep in mammals, and its associated abnormalities and their link to psychiatric disorders. The goal of the research is to uncover the neural circuits underlying REM sleep and to understand the mechanisms by which these become dysfunctional in psychiatric disorders. An essential technique for their research is to manipulate and monitor the activity of distinct neuronal cell-types. They will use AAV to deliver genes such as fluorescence markers (GFP, tdTomato), light-gated ion channels (Channelrhodopsin), and genetically encoded Calcium indicator (GCamp6). They will also use AAV for monosynaptic tracing using pseudotyped rabies virus. Pseudotyped mutant rabies virus can only propagate in genetically specified small subsets of cells in the brain expressing the B19 glycoprotein and TVA receptor as transgenes. They will inject up to 1 mL of AAV encoding B19-Glycoprotein and TVA into the target area in the brain. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

24. Yang..............24-126...........**D-1, 3 O-1**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of 3rd generation lentiviral vectors in cell culture such as IPS cells and human Mycoblast cells and has been purchased from Addgene. The lab has identified type 2 diabetes risk genes such as HMBS, VPS11 from GWAS studies (Genome-wide association studies). HMBS is an enzyme in the heme biosynthesis pathway; dietary heme intake has been linked to diabetes risk. They aim to use lentiviral vectors to increase or knock out/down the expression of these genes in vitro to interrogate gene function. They will also use overexpression and CRISPR activation/repression (dCas9-VP64 and dCas9-KRAB) for modulation of gene expression. Containment has been set to BSL-2.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

25. Yang..............24-355...........**D-1, 2**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the use of the Cytotune 2.0 Sendai Reprogramming Kit from Thermo Fisher and a plasmid which contains an Human Herpes 4 gene expression regulator, which supports the reprogramming. Patient-specific somatic cells are transduced in vitro with Sendai vectors to deliver Yamanaka factors (Sox2, Klf4, c-myc, Oct3/4) that are critical for efficient generation of iPSCs. Modified cells are provided to end users of the core who use these in a variety of in vitro applications such as differentiation and gene editing. The Sendai reprogramming vectors are based on a modified, non-transmissible form of SeV which has the Fusion protein (F) deleted, rendering the virus incapable of producing infectious particles from infected cells. These Sendai Virus (SeV) vectors do not integrate into the host genome and instead remain in the cytoplasm. The host cell can be cleared of the vectors and reprogramming factor by culturing the cells in a nonpermissive temperature (38°C) (instead of 37C). Additionally,

we will also be using non-viral (episomal replication) method to generate integration-free human iPS cells from peripheral blood cells. Containment has been set to BSL-2.

- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

27. Sundstrom....25-056...........**D-4**

- This registration was presented by Ms. Stephanie Adams-Tzivelekidis. This registration is for the generation and/or use of adeno-associated viral vectors. The lab is interested in investigating the pharmacokinetics of exogenously extracellular vesicles (EVs) introduced into the eye and their potential for use in novel ocular therapeutics. Administration of EVs from therapeutically relevant cells have been shown to enhance regeneration, lower inflammation, and increase survival of various tissues. To investigate relationship between EVs and disease in the eye and their potential as a therapy, EVs isolated from human cell culture and modified with AAV to express shRNA for gene knock down will be injected into the eyes of mice. Established and experimental therapies using AAVs will be used in the eye for comparison. The lab also requests a containment downgrade to ABSL1 for the injection of EVs, which were isolated from human iPSC differentiate into retinal pigment epithelium cells (RPE). They are utilizing a master cell bank management system, as well as adhering to BSL2 practices to ensure no re-infection of the differentiated cells, as well as exposure risks. Containment has been set to BSL-2 ABSL-1.
- The registration was discussed by committee members. Dr. Daniel Kessler requested more details of AAV use and for modified cells into animals to be selected.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved pending changes.

SECTION III-O. Experiments that Require IBC Approval Before Initiation:

26. Husbands .....25-168...........**E-2 O-1**

- This registration was presented by Dr. Sarah Capasso. This registration is for the generation and/or use of transgenic *Arabidopsis thaliana* and *Brachypodium distachyon. Agrobacterium tumefaciens* will be used to deliver plasmids carrying the genes of interest or CRISPR/Cas9 components to plants through floral dipping. A fluorescent protein will be used to monitor the accumulation of certain genes in the transgenic plant lines. Herbicide resistance will be used to select transformed plants. These plants will allow the lab to study transcription factors and developmental regulators. Containment has been set to BSL-1 and ABSL-1.
- The registration was discussed by committee members.
- Training was complete.
- All members were in favor of approval.
- The IBC registration was approved.

SECTION III-E. IBC Notice Simultaneous with Initiation

27. Sundstrom......25-056............~~**E-1**~~ **(see above)** reclassified under SECTION III-D.

3. New Business:

- No new business was scheduled.
- Dr. Dan Kessler took this opportunity to discuss the upcoming compliance responsibility regarding posting of approved IBC minutes. The minutes from this meeting, after approval during the 7-28-2025 IBC meeting will be posted to the IBC web site.
- The IBC also commented on the new abbreviated summary document provided to the committee. Because of a Microsoft security requirement, some IBC members could not utilize the form for their summaries. Dr. Andrew Maksymowych stated that he will modify the form for easier access and will provide it to IBC membership before the next meeting.

4. Old Business:

- No Old Business Scheduled.

5. End Meeting:

- The Institutional Biosafety Committee Meeting was adjourned by Dr. Daniel Kessler at **11:15 AM**.

Our next meeting scheduled for Monday, July 28th, 2025, will be held on site at the EHRS Office with a Teleconference option, at 10:00 am. A light Brunch will be provided.