

Curriculum Vitae
Paul R. Clark, Ph.D.

Current Work Address:

Department of Immunobiology
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Home Address:

57 Exchange St.
New Haven, CT 06513

Education:

1999 - Doctor of Philosophy (Ph.D.), Immunology
University of Arizona
Tucson, AZ

Advisor: Evan M. Hersh, M.D.

Dissertation Title: “Adenovirus- and cationic-lipid-mediated intratumoral gene transfer and natural killer cell activity in a SCID mouse melanoma tumor model”

1985 - Bachelor of Science (B.S.), Microbiology
University of Arizona
Tucson, AZ

References:

Kevan C. Herold, MD
Director, Yale Diabetes Center
Deputy Director, Yale Center for Clinical Investigation
Kevan.herold@yale.edu

Jordan S. Pober, MD, PhD
Director, Human and Translational Immunology Program
Vice-Chair, Dept. of Immunobiology for the Section of Human and Translational Immunology
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Paula Preston-Hurlburt, PhD
Lab Manager for Kevan C. Herold, M.D.

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Roger Babbitt
Lab Manager for William Sessa, PhD
roger.babbitt@yale.edu

Nancy Kirkiles-Smith, Ph.D.
Lab Manager for Jordan Pober, MD, PhD
nancy.kirkiles@yale.edu

Professional Experience:

Apr. 2016 – Present	Associate Research Scientist – Kevan C. Herold, MD Department of Immunobiology Yale School of Medicine New Haven, CT
Feb. 2004-Apr. 2016	Associate Research Scientist – Jordan S. Pober, MD, PhD Vascular Biology and Therapeutics Program Department of Immunobiology Yale School of Medicine New Haven, CT
Aug. 2001-Feb. 2004	Post-doctoral Fellow – Diane Krause, MD, PhD Department of Laboratory Medicine Yale School of Medicine New Haven, CT
July 1999-Aug. 2001	Post-doctoral Fellow – Antoine Menoret, PhD Center for Immunotherapy of Cancer and Infectious Diseases University of Connecticut Health Center Farmington, CT
Aug. 1994-May 1999	PhD Candidate Department of Microbiology and Immunology University of Arizona Tucson, AZ
Aug. 1994-May 1999	Graduate Research Assistant Arizona Cancer Center Tucson, AZ
July 1991-July 1994	Senior Research Technician

ISIS Pharmaceuticals, Inc.
San Diego, CA

Aug. 1988-July 1991

Research Technician
Molecular Biosystems, Inc.
San Diego, CA

Publications:

Durning SP, Preston-Hurlburt P, **Clark PR**, Xu D, Herold KC. (2016) The Receptor for Advanced Glycation Endproducts Drives T Cell Survival and Inflammation in Type 1 Diabetes Mellitus. *J Immunol*. 197(8):3076-3085.

Clark PR, Kim RK, Pober JS, Kluger MS. (2015) Tumor necrosis factor disrupts claudin-5 endothelial tight junction barriers in two distinct NF- κ B-dependent phases. *PLoS One*. Mar 27:10(3).

Kluger MS, **Clark PR**, Tellides G, Gerke V, Pober JS. (2013) Claudin-5 controls intercellular barriers of human dermal microvascular but not human umbilical vein endothelial cells. *Arterioscler Thromb Vasc Biol*. 33(3):489-500.

Clark PR, Jensen TJ, Kluger MS, Morelock M, Hanidu A, Qi Z, Tataka RJ, Pober JS. (2011) MEK5 is activated by shear stress, activates ERK5 and induces KLF4 to modulate TNF responses in human dermal microvascular endothelial cells. *Microcirculation*. 18(2):102-117.

Clark PR, Pober JS, Kluger MS. (2008) Knockdown of TNFR1 by the sense strand of an ICAM-1 siRNA: dissection of an off-target effect. *Nucleic Acids Res* 36(4):1081-1097.

Clark PR, Manes TD, Pober JS, Kluger MS. (2007) Increased ICAM-1 expression causes endothelial cell leakiness, cytoskeletal reorganization and junctional alterations. *J Invest Dermatol*. 127(4):762-774.

Kale S, Karihaloo A, **Clark PR**, Kashgarian M, Krause DS, and Cantley LG. (2003) Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. *J Clin Invest* 112(1):42-49.

Callahan M., Chaillot D., Jacquin C, **Clark PR**, and Ménoret A. (2002) Differential acquisition of antigenic peptides by Hsp70 and Hsc70 under oxidative conditions. *J Biol Chem* 277(37):33604-33609.

Clark PR and Menoret A. (2001) The inducible Hsp70 as a marker of tumor immunogenicity. *Cell Stress Chaperones* 6(2):121-125.

Clark PR, Stopeck AT, Parker SE, Hersh EM. (2000) Cationic lipid gene transfer of an IL-2 transgene leads to activation of natural killer cells in a SCID mouse human tumor xenograft. *Cell Immunol* 204(2):96-104.

Clark PR, Stopeck AT, Parker SE, and Hersh EM. (2000) Studies of direct intratumoral gene transfer using cationic lipid-complexed plasmid DNA. *Cancer Gene Ther* 7(6):853-860.

Tsang TC, Brailey JL, Vasanwala FH, Wu RS, Liu F, **Clark PR**, Meade-Tollin L, Luznick L, Stopeck AT, Akporiaye ET, and Harris DT. (2000) Construction of new amplifier expression vectors for high levels of IL-2 gene expression.

Int J Mol Med 3:295-300.

Clark PR, Stopeck AT, Brailey J, Wang Q, McArthur J, Finer MH, and Hersh EM. (1999) Polycations and cationic lipids enhance adenovirus transduction and transgene expression in tumor cells.

Cancer Gene Ther 6:437-446.

Clark PR and Hersh EM. (1999) Cationic Lipid-Mediated Gene Transfer: Current concepts. *Curr Opin Mol Ther* 1(2):158-176.

EM Hersh, Stopeck AT, and **Clark PR**. (1998) Intratumoral gene delivery for cancer treatment – an overview. *Curr Res Mol Ther* 1(5):335-338.

Stopeck AT, Hersh EM, Brailey JL, **Clark PR**, Norman J, and Parker SE (1998) Transfection of primary tumor cells and tumor cell lines with plasmid DNA/lipid complexes.

Cancer Gene Ther 5:119-126.

Clark PR, Roberts ML, and Cowser LM. (1997) A novel drug screening assay for papillomavirus-specific antiviral activity.

Antiviral Res 37:97-106.

Experience, Duties and Responsibilities:

2016-Present Associate Research Scientist – Herold Lab

1. Development and utilization of a Proximity Ligation Assay (PLA) to confirm binding of HMGB1 to RAGE (Receptor for Glycation Endproducts) in Type 1 Diabetes (T1D) patient T Cells.
 - Result: Publication of peer-reviewed study.
2. Characterization of putative RAGE agonists identified by Mass Spec-analysis of RAGE-bound proteins from T1D patient serum.
3. Use of CRISPR/Cas9 to produce RAGE-null cell models.

2004-2016 Associate Research Scientist - Pober Lab

1. Investigation of the role of ICAM-1 as a mediator of vascular permeability in human dermal microvascular endothelial cells (HDMEC).
 - Purification of human endothelial cells from donor skin using tissue disaggregation, magnetic bead positive immuno-selection and sterile cell culture techniques under BSL2 safety conditions.
 - Retrovirus (LZRS) design, construction, propagation and infection of human endothelial cells.
 - Measurement of trans-endothelial electrical resistance (TEER) to measure barrier function using a Ussing chamber.
 - Induction of cell adhesion molecules (ICAM-1 and E-selectin) using pro-inflammatory cytokines, including TNF- α . Analysis by flow cytometry, immunofluorescence (IF) and Western blot.
 - Inhibition of protein expression using siRNA.
 - Preparation and editorial review of a peer-reviewed and published manuscript.
2. Discovery and description of a sequence-specific, off-target effect by an ICAM-1-targeting siRNA whose sense-strand specifically degrades TNFR1 mRNA.
 - Endothelial cell enumeration via fluorescent dye (Hoechst) incorporation using fluorescence plate reader.
 - Design and development of a novel soluble TNFR1 enzyme-linked immunosorbent assay (ELISA).
 - Measurement of cell apoptosis and necrosis with AnnexinV antibody/propidium iodide and flow cytometry.
 - Quantitative real time polymerase chain reaction (qRT-PCR) primer design, analysis and multiplex assay development.
 - Phospho-protein analysis by Western blot.

- PCR cloning, site-directed mutagenesis and chimera construction of TNFR1 gene.
 - Design and use of chemically modified siRNA analogs.
 - Preparation and editorial review of a peer-reviewed and published manuscript.
3. Investigation of laminar shear stress in endothelial cells leading to gene activation and TNF- α response modulation.
- Immunofluorescent staining of human donor skin for multiple antigens.
 - Development of a shear stress *in vitro* flow cell assay for human endothelial cells.
 - Purification of human umbilical cord arterial and venular endothelial cells by CD31 immunoselection.
 - Hemagglutinin (HA)-tag primer design, PCR amplification, sub-cloning and retroviral transduction of an HA-tagged MEK5 cDNA into HDMEC.
 - RNA microarray analysis, and qRT-PCR confirmation, of MEK5/CA (constitutively active) transduced HDMEC.
 - PCR cloning of the KLF4 promoter region and construction of a KLF4 promoter-luciferase reporter plasmid.
 - PCR cloning of the KLF4 3'untranslated region (3'UTR) and construction of a luciferase-3'UTR reporter plasmid.
 - Preparation and editorial review, along with external collaborators, of a peer-reviewed and published manuscript.
4. Development of novel assay platforms for the study of Claudin5 in microvascular EC permeability and function.
- Adaptation of an automated, real-time Electric Cell Impedance Sensing (ECIS) instrumentation platform to monitor endothelial permeability.
 - Use of siRNA and shRNA (short hairpin RNA) in conjunction with ECIS to study cellular regulators of vascular permeability.
 - Use of site-directed mutagenesis to remove potential tyrosine phosphorylation sites in human Claudin5.
 - Design, construction, preparation and transduction of lentiviral vector supernatants expressing mutant Claudin5.
 - Immunoprecipitation with Claudin5- and phosphotyrosine-specific antibodies from wild-type and mutant Claudin5 cell lysates.
 - Development of a chromatin immunoprecipitation (ChIP) assay to determine E-selectin promoter occupancy by NF-kB transcription factors.

2001-2004 Post-doctoral Fellow – Krause Lab

1. Studies of murine stem cell transdifferentiation.
- Purification of multi-potent, lineage-negative (Lin⁻) murine bone marrow stem cells with leukocyte density centrifugation and a magnetic bead-conjugated immunoseparation assay.
 - Design and use gene-specific primers for PCR mRNA expression profiling following cytokine stimulation of murine stem cells.

2. Studies of stem cell homing in mice.
 - ¹¹¹Indium-labeling of bone marrow-derived Lin⁻ cells and single photon emission computerized tomography (SPECT) analysis to assess cellular homing.
 - Intra-splenic stem cell survival surgery of male stem cells into female and *in situ* hybridization analysis for Y chromosome presence.
 - Tail vein injection of PKH26-labeled murine stem cells and homing analysis by flow cytometry.

1999-2001 Post-doctoral Fellow – Menoret Lab

1. Discovery that heat shock protein 70 (HSP70) induction in a MethA fibrosarcoma tumor model, is necessary and sufficient, to confer protective tumor immunity.
2. Cloning and purification of mammalian HSP70 and preparation of HSP70-antigen peptide complexes as candidate tumor vaccines.
 - cDNA synthesis, design of His-tag containing amplification primers, and subcloning of His-tagged HSP70 into a bacterial protein expression vector.
 - Purification of HSP70 using Nickel-affinity column purification.
3. Evaluation of an HSP70-peptide vaccine and its ability to deliver HSP70-complexed SIINFEKL peptide as measured by OT-I cytotoxic T-lymphocyte (CTL) killing in a ⁵¹Cr-release assay.

1994-1999 Ph.D. Candidate/ Graduate Research Assistant – Hersh Lab

1. Optimization of transfection and transduction protocols for expressing therapeutic transgenes, including IL-2, using novel cationic lipids, adenovirus and lipid-virus complexes.
 - Preparation of primary tumor isolates from fresh human tumors with collagenase, DNase I and hyaluronidase disaggregation.
 - Care and maintenance of SCID (C.B-17 *scid/scid*) mouse colony and subcutaneous injection of UM449 tumor cells, injection of tumors and humane euthanasia of subject mice by accepted IACUC protocols.
 - Preparation of single-cell suspension from post-mortem UM449 tumors.
 - Maintenance of patient privacy and good laboratory practices (GLP) for storage and recording of patient samples.
 - Development and application of a colorimetric enzyme assay for β-galactosidase activity by spectrophotometry.
 - Safe handling and use of E1-deficient adenovirus for *in vitro* and *in vivo* transduction studies and determination of optimal multiplicity of infection (MOI).
 - Preparation and editorial review, along with external collaborators, of a peer-reviewed and published manuscript.

2. Optimization of intra-tumoral IL-2 expression in a subcutaneous, xenogeneic tumor model using cationic lipid-plasmid complexes in support of ongoing human clinical trials for the treatment of cutaneous melanoma.
 - Development and application of hIL-2-specific sandwich ELISA.
 - Development and application of an intracellular hIL-2-specific flow cytometric assay.
3. Study of cationic lipid-mediated, intra-tumoral IL-2 transgene expression on recruitment and activation of tumor-associated NK cells.
 - Development of Elispot immunoassay for the detection of Granzyme A exocytosis by resident tumor-associated NK cells.
 - Flow cytometric detection of intra-tumoral murine NK cells (asialoGM1⁺) and macrophages (Mac-1⁺).

1991-1994 Senior Research Technician - ISIS Pharmaceuticals, Inc.

1. Development of a novel *in vitro* human papillomavirus (HPV) replication assay.
 - HPV-11 genome purification from plasmid vector, transfection of SCC cells by electroporation and extraction of replicated forms of the HPV-11 genome from transfected cells.
 - ³²P Southern blot analysis of genome copy number.
 - E2 gene primer design and PCR confirmation of stable HPV-11 clones.
 - Determination of HPV-11 replication drug sensitivities to common anti-viral agents.
 - Use of antisense oligonucleotides to interfere with HPV-11 E2 translational start site and HPV-11 replication.
2. Development Influenza A assay platforms for the evaluation of therapeutic anti-Influenza antisense oligonucleotides.
 - Development of a methyl cellulose/crystal violet *in vitro* Influenza A viral plaque assay.
 - Development of a modified ELISA for the detection of early translation of the Influenza matrix (M) protein in Influenza infected cells in monoculture.
 - Design of therapeutic anti-Influenza A antisense oligonucleotides targeting the Influenza A matrix protein by overlapping gene-walking.
 - Analysis of antisense oligonucleotide efficacy using viral plaque assay and M protein ELISA readouts.

1988-1991 Research Technician - Molecular Biosystems, Inc.

1. Development of an oligonucleotide-based diagnostic assay for *Campylobacter jejuni*.
 - Responsibility for database maintenance of clinical samples from patients.

- Design and evaluation of methods for efficient collection of potential bacterial pathogens from clinical samples.
 - Genus specification of bacterial pathogens in clinical samples using oligonucleotide-based diagnostic probes.
 - Confirmatory analysis of results based on selective media growth.
2. Development of an oligonucleotide diagnostic assay for the detection of *Salmonella* spp. from selective growth media.
 - Maintenance of collection of reference *Salmonella* spp. serovar cultures.
 - Culture of *Salmonella* spp. on selective media, including blood agar and XLD plates.
 - Use of an oligonucleotide hybridization-based diagnostic assay for the confirmation of *Salmonella* spp. (specificity and sensitivity analysis).
 3. Development and FDA approval of a *Mycobacterium* spp. oligonucleotide diagnostic assay kit.
 - Responsibility for curating and maintaining *Mycobacterium* cultures, including *M. tuberculosis*, *M. bovis*, *M. avium* and *M. intracellulare* within a BSL3 facility.
 - Genotyping of *Mycobacterium* spp. using ribosomal RNA-specific oligonucleotide hybridization probes.
 - Design of experimental protocols in collaboration with the San Diego Department of Health to culture and identify clinical sputum *Mycobacterium* samples using Bactec, an automated and self-contained culture system.
 4. Development of an automated, *in situ* hybridization detection and genotyping assay.
 - Optimization and validation of human papilloma virus (HPV) type-specific oligonucleotide probes.
 - Design of HPV E6 region type-specific PCR primers for the amplification and sub-cloning of HPV type 6, 11, 16, 18, 31, and 33 amplicons for positive control production.
 - Sample preparation, optimization and genotyping of human cervical smears.

U.S. Patents:

“Selective modulation of tumour necrosis factor receptors in therapy”.

Inventors: John Bradley, Jordan Pober, **Paul Clark**, Wang Min, Martin Kluger

WO 2006109044 A2

Date of issue, Oct 19, 2006

”A novel drug screening assay for papillomavirus-specific antiviral activity”.

Inventors: Lex M Cowser and **Paul R Clark**

US 5821050 A

Date of issue, Oct.1998.

Knowledge, Skills and Abilities - General:

Editorial

Proven ability to research, draft and edit scientific communications, including, grant proposals, articles, reviews and regulatory reports.

Interpersonal Skills

Excellent ability to communicate and build consensus with managers, peers and junior team members.

Proven record of i) providing thoughtful and actionable advice to managers, ii) building collaborative relationships and consensus among peers, and iii) providing mentorship and constructive advice to junior researchers, research fellows and students within the institution.

Knowledge, Skills and Abilities – Biomedical:

Animal Husbandry (Murine)

1. Care and feeding
2. Eye bleeds
3. Immunologic genotyping
4. Injection (intra-peritoneal, intra-dermal, intra-muscular, tail-vein, etc.)
5. Dissection and tissue harvest (lymph node, spleen, etc.)
6. Additional invasive procedures; cardiac puncture blood draw, perfusion, etc.
7. Survival surgery (intra-splenic injection)

Radioisotopes

1. ^3H thymidine proliferation assay
2. ^{32}P DNA probe preparation
3. ^{51}Cr cell-labeling and analysis
4. ^{111}In cell-labeling and *in vivo* tail-vein injection

Microscopy

1. Dissecting microscope
2. Inverted phase contrast microscope
3. Epifluorescent microscope
4. Confocal microscope

Chromatography

1. Ion-exchange
2. Size exclusion

3. Affinity

Histochemistry and Immunohistochemistry

1. Hematoxylin/Eosin analysis of tissues including blood, skin, spleen and lymph nodes
2. Immunofluorescent and immunoperoxidase detection of cellular antigens in multiple tissues including skin, placenta, myocardium, spleen, and lymph nodes

Biophysical Separation Techniques

1. Cesium chloride gradient ultracentrifugation
2. Percoll ultracentrifugation
3. Discontinuous sucrose ultracentrifugation
4. Oligonucleotide purification by acrylamide gel electrophoresis

Cell Purification

1. Lymphocyte purification by density centrifugation (Lympho-Prep)
2. Positive and negative immune-selection (antibody-conjugated magnetic beads)
3. Fluorescent immune-sorting by FACS
4. Arterial and venous endothelial cell (EC) purification from human umbilical cords

In vitro Cell Culture

1. Propagation, sub-culture and storage of mammalian cells
2. Transfection of cells with antisense oligonucleotides, siRNA and miRNA
3. Transduction of cells with retroviral and lentiviral viral vectors
4. Viral plaque assay
5. Single-cell cloning by limiting dilution and cloning cylinders

DNA Analysis and Modification

1. Plasmid DNA propagation and purification
2. Mitochondrial DNA purification
3. Genomic DNA purification
4. DNA fragment sub-cloning (restriction endonuclease digest, fragment purification, ligation and antibiotic selection and screening)
5. Recombinant lentivirus and retrovirus design, preparation and purification
6. Site-directed mutagenesis of DNA plasmids
7. Experience with multiple web-based molecular biology and resources, including PubMed, BLAST, UniProt, and Ensembl.

RNA Analysis

1. Northern blot
2. Purification of total RNA from blood and splenocytes, cultured cells, and various tissues
3. Gene-specific primer design and qRT-PCR (Sybr-Green and TaqMan)

miRNA Analysis and Transfection

1. Purification and qRT-PCR analysis of miRNA

2. Cationic-lipid transfection of chemically synthesized miRNA

Immunoassays

1. ELISA
2. Western Blot
3. Immunoprecipitation
4. Chromatin Immunoprecipitation (ChIP)
5. Elispot
6. Flow Cytometry

Specialized Techniques

1. ECIS (Electric Cell Impedance Sensing): Measurement of trans-electrical endothelial resistance
2. Recirculating flow-cell analysis of hemodynamic shear on endothelial cells (EC)

Knowledge, Skills and Abilities - Microbiology:

Bacteriology

1. Microaerophilic culture of *Campylobacter* spp. (including *C. jejuni*).
2. Culture and maintenance of a gram-negative, Enterobacteriaceae culture collection, including *Escherichia*, *Shigella* and *Salmonella*.
3. Culture and maintenance of *Mycobacterium* spp. culture collection, including *M. bovis*, *M. tuberculosis*, *M. avium* and *M. intracellulare*.
4. Familiarity with, and practical use of, anti-bacterial antibiotics, including ampicillin, kanamycin and tetracycline.

Virology

1. Development of an *in vitro* human papillomavirus (HPV) replication assay.
2. *in vitro* plaque assay development of an infectious, replication-competent influenza virus (A/Puerto Rico/8).
3. *in vivo* assay development of a replication-deficient Adenovirus (intra-tumoral injection).
4. Design, construction, and purification of recombinant, replication-deficient retroviral expression vectors (pLZRS).
5. Design, construction, and purification of recombinant, replication-deficient lentiviral expression vectors.
6. Serologic detection of Influenza virus matrix protein by a modified cell-based enzyme-linked immunosorbant assay (ELISA).
7. Familiarity with, and practical use of, anti-viral compounds including ribavirin, 5-fluorouracil (5-FU) and 5-bromo-2-deoxyuridine (BrdU).

Toxicology

1. Extensive practical and theoretical knowledge of eukaryotic toxins, including;
 - General metabolic inhibitors: Cyclohexamide, Actinomycin
 - G protein-coupled receptor (GCPR) inhibitors: Pertussis toxin

- Pro-apoptotic compounds: Ceramide, Sphingosine-1-phosphate
- Anti-apoptotic compounds: ZVAD (caspase inhibitor)
- Immunosuppressive compounds: Cyclosporin A
- Anti-fungal compounds: Amphotericin B

Biological Safety Level 3 Training

1. *Mycobacterium tuberculosis*
2. Human Immunodeficiency Virus (HIV)

Knowledge, Skills and Abilities – Safety and Regulatory:

1. Comprehensive compliance training including, Bloodborne Pathogen & Biosafety, Chemical Hazards, Radiation Safety, and Animal Care and Treatment at 5 separate research institutions.
2. Drafting and submission of animal protocols to Institutional Animal Care and Use Committees (IACUC).
3. Drafting and submission of protocols requiring radioisotope usage to Environmental Health and Safety (EHS) committees.