Curriculum Vitae

James D. Orth

Assistant Research Professor &
Director, Light Microscopy Facility

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin – Eau Claire Eau Claire, WI	BS, *Cum laude*	05/1995	Biochemistry and Molecular Biology
Mayo Clinic College of Medicine, Rochester, MN	-	-	Masters in Tumor Biology
Mayo Clinic College of Medicine, Rochester, MN	PhD	05/2005	Cell Biology and Genetics
Harvard Medical School, Boston, MA	Post-doc	08/2012	Cancer Cell Biology, Molecular Pharmacology

A. Personal Statement

My research is focused on understanding the mechanisms of anti-cancer therapeutics using dynamic fluorescent reporters and longitudinal, single cell approaches that I pioneered as a post-doctoral fellow. I use long-term live-cell microscopy and extend some work to *in vivo* models using specialized intravital microscopy and two-photon approaches. In my research career, I've successfully developed and executed innovative projects focused on multiple mitosis-targeted therapies and targeted therapies that exploit the cell cycle. I am an academic with >20 years of research experience focused in cell biology, cell cycle, DNA damage, cell death signaling and anti-cancer therapeutics. I am located at the Molecular, Cellular, and Developmental Biology department at the University of Colorado Boulder, and am an Affiliate Member of the University of Colorado Cancer Center. In the cancer center, I bridge the cancer cell biology and developmental therapeutics programs and I specialize in basic science and preclinical studies of developmental and clinical stage anti-cancer drugs. My entire research platform easily moves between *in vitro* and *in vivo* approaches, but many of my long-term questions can only be answered using intravital microscopy, including the role of tumor cell drug-induced senescence in therapeutic response, mechanisms constituting the proliferation rate paradox, and the interaction and impact of immune system cells on tumor cells during therapeutic response.

B. Positions, Honors, Teaching and Mentoring, Service, Presentations

Positions:

06/94 – 08/94 Howard Hughes Summer Undergraduate Research Fellowship, Dept. of Molecular Medicine, Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI. Advisor: Anton Scott Goustin, Ph.D.

06/95 – 05/99 Research Technologist, Dept. of Biochemistry and Molecular Biology, Mayo Clinic of Medicine, Rochester, MN. Advisor: Jeffrey L. Salisbury, Ph.D.

09/99 – 12/99 Teaching Assistant, Mayo Clinic Graduate School, Mayo Clinic College of Medicine, Rochester, MN. Course: 'Genome Biology'.

09/00 – 12/00 Teaching Assistant, Mayo Clinic Graduate School, Mayo Clinic College of Medicine, Rochester, MN. Course: 'Chemical Principles of Biological Systems'.

06/99 – 03/05	Graduate Student, Dept. of Biochemistry and Molecular Biology, Mayo Clinic of Medicine, Rochester, MN. Advisor: Mark A. McNiven, Ph.D.
03/05 – 08/12	Research Fellow, Dept. of Systems Biology, Harvard Medical School, Boston, MA. Advisor: Timothy J. Mitchison, Ph.D.
08/12 – present	Assistant Research Professor, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO.
08/16 – present	Director, Light Microscopy Facility, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO.

Honors, Awards:

09/01	Travel Award, Keith R. Porter Symposium on Cytoplasmic Organization and Membrane Traffic, 2001. Airlie, VA.
12/02	Travel Award, ASCB annual meeting, San Francisco, CA.
2003 – present	Journal cover and other images. Covers: Mol Cancer Ther, 2008 Nov;7(11):3480-9; Curr Opin Cell Biol, 2003 Feb;15(1):31-9; Mol Biol Cell, 2003 Mar;14(3):1085-96. Contents page image, Nat Rev Mol Cell Biol, 2004 Aug;5(8).
2004	Poster of Distinction Award, Digestive Disease Week meeting. New Orleans, LA, USA
2004	Mayo Clinic Medical Collection Contributor, Mayo Clinic College of Medicine. *Dynamin and Cytoskeletal-dependent Membrane Processes*, p 189-99, Cell Motility: From Molecules To Organisms, *John Wiley & Sons, Ltd*, 2004.
08/11	Olympus BioScapes Digital Imaging Competition, Honorable Mention Award; “Live Imaging of Mitosis in Tumors”.
12/12	Faculty of 1000 Manuscript of Special Significance, Orth JD, et al., “Analysis of mitosis and anti-mitotic drug responses in tumors by in vivo microscopy and single cell pharmacodynamics”. Cancer Res. 2011 Jul 1;71(13):4698-16. PMC3129392.
09/17	Named new Excelitas LED illuminator, “X-Cite FIRE” (resulted in 50% discount on industry leading LED for fluorescent microscope)
10/17	Arts and Sciences Fund for Excellence, $1,000 award for conference travel
08/19	Front page digital image, Angarus Therapeutics; https://www.angarustherapeutics.com/

Teaching:

09/12 – present	MCDB 6440, Special Topics in MCD Biology (roughly annually)
12/12 – present	MCDB 6000, Introduction to laboratory methods, (approximately 8)
01/13 – present	MCDB 4840, Upper Division Independent Study (5 students total)
01/13 – 2018.	MCDB Graduate Student Core class
08/13	Advanced Course in Light Microscopy, University of Colorado Boulder, Boulder CO. (Instructor)
01/14 – present	MCDB 3150, Biology of the Cancer Cell (fall semesters)
04/16	CHEM 5801, Advanced Signal Transduction and Cell Cycle Regulation, two lectures
11/16	Responsible Conduct of Research, campus-wide course for NIH-supported trainees
01/20 –	MCDB 2350, Understanding Cancer: Understanding Cancer: introduction to the disease’s biology, medicine, and societal implications (spring semesters)

Mentoring and Outreach:

10/12 – present	Thesis Advisory Committee member, 10 total committees, 5 active.
2013 – present	Undergraduate Research Opportunity, UROP (3 students)
2013 – present	Research Volunteers (8 students total); at least 3 now in graduate school
2013 – present	Undergraduate Independent Study students; 6 total, 1 active
2014 – present	Biological Undergraduate Research Skills & Training, BURST (1 student)
2014 – 2018	Thesis Advisor: Mr. Russell Burke, MCDB, ‘Nuclear Export as an anti-cancer target': 1) Signaling and Cell Regulation training grant; 2) Cancer Center Annual Retreat poster; 3) CSHL course, Quantitative Imaging: From Cells to Molecules, April 2015, Helmsley Scholarship; 4) AACR Annual meeting, 2015 (poster). 5) 3 first author papers, 2 second author papers; 5) Post-doctoral fellowship, Professor Anna Huttenlocher, University of Wisconsin Madison
05/15	Crestview Elementary School Science Fair, Interviewer

06/15 – 08/15 Summer Multicultural Access to Research Training (SMART) student (Ms. Sarah Seto)
05/16 MCDB Honors Undergraduate Student, *Suma cum laude* (Ms. Andrea Doak)
05/18 Centennial Middle School 'Interview Someone with Your Future Career', Interviewee

Service, Memberships, and Other Experience:

1995 – 2012 American Society for Cell Biology member (most years).
2002 – present Peer Reviewer: Pharmacology, Communications Biology, Cancer Research, Cancers, Scientific Reports, Molecular Cancer Therapeutics, Genome Research, Cell Cycle, Nature Communications, Cancer Letters, etc.
09/12 – 3/14 Committee on Graduate Student Qualifying Examination, MCDB Graduate Program, University of Colorado Boulder
12/12 Grant internal peer reviewer, BioFrontiers Institute, University of Colorado Boulder
12/12 – 12/17 Full Member, American Association for Cancer Research
12/12 – present Affiliate Member, Cell Cycle Regulation and Proliferation Focus Group, University of Colorado Cancer Center, Aurora, CO
02/13 – present Light Microscopy Core Facility Advisory Council, University of Colorado Boulder
07/13 – present Institutional Biosafety Committee, University of Colorado Boulder
08/13 Instructor, Advanced Course in Light Microscopy, University of Colorado Boulder
08/13 External peer reviewer, grant submitted to Aix-Marseille University Excellence Initiative, "Modeling pharmacology of tubulin drugs"
03/14 – 06/16 Light Microscopy Facility Faculty Proctor, MCDB Dept., University of Colorado Boulder
08/14 – present Training Faculty, Signaling and Cellular Regulation NIH T32 GM08759 (PI: Natalie Ahn)
09/14 – 15 Committee on Graduate Student Admissions/Advising (COGSA), MCDB Dept. University of Colorado Boulder
09/15 – 2017 Graduate Student Journal Club Committee (advisory role)
03/17 – present Editorial Board Member, Scientific Reports, Nature Publishing Group.
05/17 Advisory panel for Cambridge Healthtech Institute's Single Cell Analysis Conference, February, 2018. San Francisco, CA.
03/18 Advisory panel, 4th Annual Next Generation Sequencing and Clinical Diagnostics & Single Cell Analysis USA Congress, October, 2018. Boston, MA.
05/18 External peer reviewer, grant submitted to Breast Cancer Now (UK), "Targeting therapy-induced senescence"

Presentations, Meetings:

12/95 – 2012 ASCB, BSCB, GRC, etc. as a technician, graduate student, and fellow
03/11 NIH Advanced Training Course: Frontiers in Stem Cells and Cancer. Washington D.C. (invited seminar)
05/11 Dartmouth Medical School, Norris Cotton Cancer Center – Molecular Mechanisms Seminar Series. Hanover, NH. (invited seminar)
06/11 International Conference On The Systems Biology of Human Disease. Boston, MA. (invited seminar)
04/12 Hollings Cancer Center, Medical University of South Carolina, Dept. of Developmental Cancer Therapeutics, Charleston, SC. (invited seminar)
10/12 Signaling and Cell Regulation Super Group, University of Colorado Boulder, Boulder, CO. (invited seminar)
11/12 MCDB Mentors Club, University of Colorado Boulder, Boulder, CO. (invited seminar)
06/13 Developmental Therapeutics, University of Colorado Cancer Center, held in Boulder, CO
10/13 Molecular Target and Cancer Therapeutics (AACR) meeting, Boston, MA. (poster)
10/13 Karyopharm Therapeutics, Inc. Natick, MA (invited seminar)
11/13 Cell Cycle Regulation and Proliferation Focus Group, University of Colorado Cancer Center, Aurora, CO. (invited seminar)
01/14 Molecular Oncology sub-group meeting, University of Colorado Cancer Center, held in Boulder, CO
03/14 Developmental Therapeutics/Molecular Oncology joint retreat, University of Colorado Cancer Center, Aurora, CO
09/14 Annual Retreat, "New Model Systems", University of Colorado Cancer Center, Aurora, CO. (invited seminar)

01/15 – current MCDB Graduate Student Symposium, University of Colorado Boulder, Boulder, CO
03/15 Developmental Therapeutics/Molecular Oncology joint retreat, University of Colorado Cancer Center, Aurora, CO
03/15 ANDOR Academy, Technical Seminars and Workshop, BioFrontiers Institute, Boulder, CO
04/15 American Association for Cancer Research annual meeting, Philadelphia, PA. (poster)
04/15 Karyopharm Therapeutics, Inc., Philadelphia, PA. (invited seminar)
04/16 American Association for Cancer Research annual meeting, New Orleans, LA. (poster)
10/17 3rd Annual Single Cell Analysis USA Congress, Boston, MA (invited seminar)
07/18 Harvard Medical School, "Small molecules, single cell phenomics, and anti-cancer response", Boston, MA. (July 21, invited seminar)
10/18 Joint CSD/MCDB retreat, "Phenomics to understand anti-cancer drug mechanism" (selected from abstracts)

C. Light Microscopy Facility activities

Training, Instruction, Mentoring:

- Training and instruction nearly weekly: transmitted light microscopy (brightfield, phase-contrast, DIC), epifluorescence, spinning disk confocal, laser scanning confocal, structured illumination, specialized live cell imaging, 96 well plate screening, large area montaging, 3D imaging (tissues, organoids), multi-photon; 9 instruments; 5 acquisition softwares; data management, data analysis and quantification; image processing
- Teach users basics of light microscopy, sample preparation, technical considerations, image analysis, basic cell biology, project relevant information when appropriate. Mentoring is a component of the facility that is solicited by the users often regarding project, career plans, course options, etc.
- Current user group is >120 individuals from at least 6 departments on campus
- External users have included: University of Wyoming, University of Colorado Medical School, University of Colorado Denver
- Commercial clients (multiple)
- Acknowledged in numerous peer-reviewed publications resulting from facility activity
- Invited Moderator position, Microlist.org microscopy form.

Management:

- Supervisor of Microscopy Facility specialist, Mr. Joaquin Quintana
- Facility web page redesign and development
- Meeting with RIO, IT and other offices to discuss core facility development and evaluate and select new facility management software (Stratacore)
- Monthly billing including tracking of hours per user, hours per investigator, hours per instrument, and other revenue flow statistics; typical revenue is $6-9000/month
- Develop and implement policy of the facility: access, high-volume usage, long-term timelapse, SOP for use of mammal and primate-derived live samples, etc.
- Microscope maintenance: Carl Zeiss CLSM510 RTC replacement; spinning disk confocal microscope critical alignment; cleaning of optical paths; stage micrometer confirmation of resolution; measure laser power outputs; point spread function using 100nm beads; diagnose and clean light path; focus 405nm FRAP laser; lamp replacement and alignment; coordinate service visits when needed; general cleaning of surfaces to minimize dust
- Computer maintenance: Includes all PCs associated with microscopes and 2 stand-alone workstations
- Facility tours to prospective faculty, graduate students, and potential donors
- Liaison with many microscope, digital imaging and associated companies; Leica, Nikon, Olympus, Carl Zeiss, 3i, GE Healthcare, Sutter, Keyence, ibidi, Greiner Bio-One, Chroma, etc.

Resource Use and Implementation:

- MCDB undergraduate laboratory (Alison Vigers)
- iGEM students (Brian DeDecker)
- Interact with EM facility and users to assist with CLEM
- Help Investigators with minor microscope maintenance, e.g. cleaning; alignment; bulb replacement
- Pilot testing of experiment feasibility and optimization of work flow

- Instrument descriptions and capabilities for grant applications
- Special images for grants or presentations; new approach development and testing, e.g. FRAP, experimental design and trouble-shooting, sample preparation, etc.
- Commercial contracted imaging, including specialized
- Microscope updating: piezo z-stage; new objectives; software updates; new color camera for brightfield imaging; new glass for novel wavelengths; custom optical pathways; assisted SIM; TIRF
- Computer improvements; SSDs; displays; network access and performance; software updates, etc.

Resource Development and Acquisition:

- Seminars: 2017: Olympus VS120 Virtual Slide Scanner, "Whole Slide Imaging; Digitally Scan Entire Slides"; Yokogawa/Olympus CV1000, "Spinning Disk Confocal Microscope"; 2018: Carl Zeiss, "LSM880 Airyscan 2P Confocal". Olympus – 'What is 2-photon microscopy and what can your new FVMPE-RS do for you?'; Olympus "The power of bioluminescence microscopy"; Leica – 'What is light sheet microscopy and when should you use it?'; Stephanie Meyer (Anshutz), 'What is the latest in in vivo super resolution STED microscopy'?; 2019: Leica – STED; Imaris – Digital image analysis
- Workshops and equipment demonstrations: Olympus VS120 Virtual Slide Scanner; Yokogawa/Olympus CV1000; Excelitis LED; Zeiss LSM880 with Airyscan and 2-photon; Olympus FVMPE-RS 2-photon systems; Nikon TiE2 expanded field WF, deconvolution, color camera; Leica SP8 LSCM/STED; Leica fluorescent/color stereoscope; Keyence automated widefield/SIM; Planned in 2020 Olympus α3 lightsheet or Olympus Spin SR, Olympus FV3000 STED, Leica stereoscope with Thunder
- Negotiated purchase of Yokogawa/Olympus CV1000; includes 5 years of service
- Negotiated purchase of Olympus FVMPE-RS 2 photon microscope; includes offline Imaris with workstation and 5 years of service on Spectra Physics lasers and Olympus system
- With Investigators, plan to implement ultrastructure expansion microscopy (U-ExM) using our Nikon structured illumination microscopy instrument.

Equipment Grants:

- Please see Grants section at end of CV

D. Contribution to Science

Longitudinal microscopy to study single cell pharmacodynamics during anti-cancer therapy

Anti-cancer drug action is traditionally studied using static approaches that measure population averages and fixed cell end-points, and mechanisms and responses are oversimplified. I developed a long-term longitudinal approach using the microscope and study directly how cells respond to anti-mitotic drugs. Using this approach, I discovered profound cell-to-cell heterogeneity in response and cell fate within a single cell population and between cell types and that cancer-derived cell lines harbor a strong mitotic checkpoint equal to or greater than non-transformed cells. Unexpected cell cycle-associated cell fates with experimental therapeutics were also defined and cancer cells are generally more prone to death although cell cycle arrest responses are highly related.

1. Shi J, **Orth JD**, Mitchison TJ. Cell type variation in responses to antimitotic drugs that target microtubules and kinesin-5. *Cancer Res*. 2008 May 1;68(9):3269-76. PMID: 18451153.
2. **Orth JD**, Tang Y, Shi J, Loy CT, Amendt C, Wilm C, Zenke FT, Mitchison TJ. Quantitative live imaging of cancer and normal cells treated with Kinesin-5 inhibitors indicates significant differences in phenotypic responses and cell fate. *Mol Cancer Ther*. 2008 Nov;7(11):3480-9. PMID: 18974392.
3. Huang HC, Shi J, **Orth JD**, Mitchison TJ. Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. *Cancer Cell*. 2009 Oct 6;16(4):347-58. PMID: 19800579.
4. Burke RT and **Orth JD**. Through the looking glass: time-lapse microscopy and longitudinal tracking of single cells to study anti-cancer therapeutics. *J Vis Exp*. 2016 May 14;(111). PMID: 27213923.

Stress pathways, DNA damage and p53 dynamics after mitosis- and nuclear export-targeted anti-cancer therapies

Genotoxic stress occurs after many anti-cancer treatments, this was unclear for mitosis-targeted drugs due to indirect assays and a lack of single cell longitudinal measures. I found that late during mitotic arrest, cells partially execute apoptosis resulting in the activation of caspase-activated DNAse and subsequent DNA damage which contributes significantly to p53 induction and potent cell cycle arrest in the surviving population.

I measured p53 levels and dynamics in individual live cells and found unique p53 signatures. This occurs for multiple mitotic-targeted drugs in cell lines from different tissue origins, indicating the underlying molecular mechanism is conserved, providing a strong rationale for developing new drug combination approaches.

1. **Orth JD**, Loewer A, Lahav G, Mitchison TJ. Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. *Mol Biol Cell*. 2012 Feb 15;23(4)567-76. PMID: 22171325.
2. Park D, Burke RT, Marcus JM, and **Orth JD**. Forced nuclear retention of p53 using nuclear export inhibitors to enhance cancer cell killing. In preparation.

In vivo pharmacodynamics of mitosis-targeted anti-cancer therapies using fluorescent biosensors

The responses of cancer cells to therapies are determined at the molecular and cellular level. A significant challenge is to develop approaches that allow us to study molecular mechanism and cell behavior in situ. To pursue this, I created multiple fluorescent xenograft models and have collaborated with Drs. Tim Mitchison, Ralph Weissleder and Gaudenz Danuser. Using intravital microscopy, mitosis, apoptosis, cell cycle progression and drug-associated cell death were followed longitudinally in real time in xenograft tumors over a period of weeks. The same positions within tumors can be imaged at high time resolution to capture transient and rare events, and at the same time the entire tumor is imaged. I discovered that despite a low mitotic index, xenograft tumors responded nearly completely with a mitosis-targeted drug, paclitaxel, indicating the proliferation rate paradox, which was further supported by in vivo cell cycle profiling. Intravital microscopy serves as a paradigm to study therapeutic response, cell and tumor-based mechanisms, and can be used to study cancer relapse and drug resistance, metastasis, and systems pharmacology.

1. **Orth JD**, Kohler RH, Foijer F, Sorger PK, Weissleder R, Mitchison TJ. Analysis of mitosis and anti-mitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res*. 2011 Jul 1;71(13):4698-16. PMID: 21712408.
2. Laughney AM, Kim E, Sprachman, MM, Miller MA, Kohler RH, Yang KS, **Orth JD**, Mitchison TJ, and Weissleder R. Single-cell pharmacokinetic imaging reveals a therapeutic strategy to overcome drug resistance to the microtubule inhibitor eribulin. *Sci Transl Med*. 2014 Nov 5;6(261). PMID: 25378644.
3. §Chittajallu DR, §Florian S, Kohler RH, **Orth JD**, Sorger PK, Weissleder R, Danuser G, Mitchison TJ. In vivo cell cycle profiling in xenograft tumors by quantitative intravital microscopy. *Nat Methods*. 2015 Jun;12(6):577-85. PMID: 25867850.

Nuclear export as a cancer target, mechanisms of action and single cell phenomics

Selective inhibitors of nuclear export (SINE) target exportin-1 nuclear export and are an important new class of anti-cancer agents. The anti-cancer effects of inhibition of nuclear export are unclear. We applied our expertise and tools to begin to understand the action SINE on individual cells. Using multiple cell models, we created we have studied a correlated cell cycle, DNA damage, nucleolar stress, and cell death response. We propose that inhibition of nuclear export in cancer cells in G1- and early S-phase potently arrests and/or kills them, while cancer cells treated in G2-phase progress through mitosis first, before showing drug effects in the subsequent G1-phase; nuclear export is blocked in all cell cycle stages. Significant DNA damage in G1-phase in several cancer cell lines correlates with stronger cell cycle arrest and cell death – but DNA damage does not occur in two non-transformed cell models. The DNA damage is concomitant with nucleolar stress and is RNA polymerase I-dependent. This work paves the way to deep mechanistic studies and rational drug combinations with SINE.

1. Marcus JM, Burke RT, DeSisto J, and **Orth JD**. Longitudinal tracking of single live cancer cells to understand cell cycle effects of the nuclear export inhibitor, selinexor. *Sci Rep*. 2015 Sep 24;5:14391. PMID: 26399741.
2. Burke RT, Marcus JM, and **Orth JD**. Inhibition of exportin-1 function results in rapid cell cycle-associated DNA damage in cancer cells. *Oncotarget*. 2017 Apr 12. PMID: 28467801.
3. Marcus JM, Burke RT, Doak AM, Park S, and **Orth JD**. Loss of p53 expression alters cell cycle response to inhibition of nuclear export, but does not prevent cell death. *Cell Cycle*. 2018 July 23. PMID: 30037299.
4. Burke RT, Marcus JM, Bai S, Park D, and **Orth JD**. Nucleolar stress response concomitant with DNA damage after inhibition of exportin-1. *Sci Rep*. In revision.

Complete List of Published Work:

Total published and in revision: 30. First author: 8. Articles: 22. Review/Technical: 5. Book chapters: 1. In revision: 1. Submitted: 1. In preparation: 1.

ORCID ID https://orcid.org/0000-0002-8559-6186
Scopus Author ID 7007052206

All Publications (newest to oldest)

1. Davis SL, Ionkina A, Bagby SM, Marcus JM, **Orth JD**, Lam ET, Corr BR, O'Bryant CL, Glode A, Tan AC, Kim J, Tentler JJ, Capasso A, Dailey K, Gustafson DL, Messersmith WA, Leong S, Eckhardt SG, Pitts TM, and Diamond JR. The Combination of TORC1/2 Inhibitor TAK-228 and Aurora A Kinase Inhibitor Alisertib as a New Therapeutic Strategy in Solid Tumors: Preclinical and Dose-Finding Phase I Trial Results. *Clin Can Research.* In revision.
2. Burke RT, Marcus JM, Bai S, Park D, and **Orth JD**. Nucleolar stress response concomitant with DNA damage after inhibition of exportin-1. *Sci Rep*. In revision.
3. Fu X, Nahar A, Polovin G, **Orth JD**, and Park S. Novel Stress Responses Mediated by Multiple ATPase Subunits during Chaperone-dependent Proteasome Assembly. *J Biol Chem*. 2019 Apr 19;294(16):6562-77. PMID: 30814255.
4. Marcus JM, Burke RT, Doak AM, Park S, and **Orth JD**. Loss of p53 expression alters cell cycle response to inhibition of nuclear export, but does not prevent cell death. *Cell Cycle*. 2018 July 23; 17(11):1329-1344. 17(11):1329-1344. PMID: 30037299.
5. Burke RT, Marcus JM, and **Orth JD**. Inhibition of exportin-1 function results in rapid cell cycle-associated DNA damage in cancer cells. *Oncotarget*. 2017 Jun 13;8(24):39460-75 12. PMID: 28467801.
6. Burke RT and **Orth JD**. Through the looking glass: time-lapse microscopy and longitudinal tracking of single cells to study anti-cancer therapeutics. *J Vis Exp*. 2016 May 14;(111). PMID: 27213923.
7. Marcus JM, Burke RT, DeSisto J, and **Orth JD**. Longitudinal tracking of single live cancer cells to understand cell cycle effects of the nuclear export inhibitor, selinexor. *Sci Rep*. 2015 Sep 24;5:14391. PMID: 26399741.
8. §Chittajallu DR, §Florian S, Kohler RH, **Orth JD**, Sorger PK, Weissleder R, Danuser G, Mitchison TJ. In vivo cell cycle profiling in xenograft tumors by quantitative intravital microscopy. *Nat Methods*. 2015 Jun;12(6):577-85. PMID: 25867850.
9. Laughney AM, Kim E, Sprachman, MM, Miller MA, Kohler RH, Yang KS, **Orth JD**, Mitchison TJ, and Weissleder R. Single-cell pharmacokinetic imaging reveals a therapeutic strategy to overcome drug resistance to the microtubule inhibitor eribulin. *Sci Transl Med*. 2014 Nov 5;6(261):261ra152. PMID: 25378644.
10. **Orth JD**, Loewer A, Lahav G, Mitchison TJ. Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. *Mol Biol Cell*. 2012 Feb 15;23(4)567-76. PMC3279386.
11. **Orth JD**, Kohler RH, Foijer F, Sorger PK, Weissleder R, Mitchison TJ. Analysis of mitosis and anti-mitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res*. 2011 Jul 1;71(13):4698-16. PMC3129392.
12. Tang Y, **Orth JD**, Xie T, Mitchison, TJ. Rapid induction of apoptosis during Kinesin-5 inhibitor-induced mitotic arrest in HL60 cells. *Cancer Lett*. 2011 Nov 1;310:15-24. PMC3155259.
13. Huang HC, Shi J, **Orth JD**, Mitchison TJ. Cell death when the SAC is out of commission. *Cell Cycle*. 2010 Jun 25;9(11):2049-50. PMID: 20559025.
14. Tsui M, Xie T, **Orth JD**, Carpenter AE, Rudnicki S, Kim S, Shamu CE, Mitchison TJ. An intermittent live cell imaging screen for siRNA enhancers and suppressors of a kinesin-5 inhibitor. *PLoS One*. 2009 Oct 5;4(10):e7 339. PMC2752188.
15. Huang HC, Shi J, **Orth JD**, Mitchison TJ. Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. *Cancer Cell*. 2009 Oct 6;16(4):347-58. PMC2758291.
16. **Orth JD**, Tang Y, Shi J, Loy CT, Amendt C, Wilm C, Zenke FT, Mitchison TJ. Quantitative live imaging of cancer and normal cells treated with Kinesin-5 inhibitors indicates significant differences in phenotypic responses and cell fate. *Mol Cancer Ther*. 2008 Nov;7(11):3480-9. PMC2597169. Cover article.
17. Shi J, **Orth JD**, Mitchison TJ. Cell type variation in responses to antimitotic drugs that target microtubules and kinesin-5. Cancer Res. 2008 May 1;68(9):3269-76. PMID: 18451153.
18. **Orth JD**, McNiven MA. Get off my back! Rapid receptor internalization through circular dorsal ruffles. *Cancer Res*. 2006 Dec 1;66(23):11094-6. PMID: 17145849.
19. **Orth JD**, Krueger EW, Weller SG, McNiven MA. A novel endocytic mechanism of epidermal growth factor receptor sequestration and internalization. *Cancer Res*. 2006 Apr 1;66(7):3603-10. PMID: 16585185.
20. Cao H, Weller S, **Orth JD**, Chen J, Huang B, Chen JL, Stamnes M, McNiven MA. Actin and Arf1-dependent recruitment of a cortactin-dynamin complex to the Golgi regulates post-Golgi transport. *Nat Cell Biol*. 2005 May;7(5):483-92. PMID: 15821732. Comment in: *Nat Cell Biol*. 2005 May;7(5):448-9, "Extending the court for cortactin: from the cortex to the Golgi". PMID: 15867926.

21. Yao Q, Chen J, Cao H, **Orth JD**, Michael McCaffery J, Stan RV, McNiven MA. Caveolin-1 interacts directly with dynamin-2. *J Mol Biol*. 2005 Apr 29;348(2):491-501. PMID: 15811383.
22. Buccione R, **Orth JD**, McNiven MA. Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. *Nat Rev Mol Cell Biol*. 2004 Aug;5(8):647-57. PMID: 15366708.
23. Chen XM, Huang BQ, Splinter PL, **Orth JD**, Billadeau DD, McNiven MA, LaRusso NF. Cdc42 and the actin-related protein/neural Wiskott-Aldrich syndrome protein network mediate cellular invasion by Cryptosporidium parvum. *Infect Immun*. 2004 May;72(5):3011-21. PMC387898.
24. §Krueger EW, §**Orth JD**, Cao H, McNiven MA. A dynamin-cortactin-Arp2/3 complex mediates actin reorganization in growth factor-stimulated cells. *Mol Biol Cell*. 2003 Mar;14(3):1085-96. §A co-first author manuscript. PMC151581.
25. Cao H, **Orth JD**, Chen J, Weller SG, Heuser JE, McNiven MA. Cortactin is a component of clathrin-coated pits and participates in receptor-mediated endocytosis. *Mol Cell Biol*. 2003 Mar;23(6):2162-70. PMC149460.
26. **Orth JD**, McNiven MA. Dynamin at the actin-membrane interface. *Curr Opin Cell Biol*. 2003 Feb;15(1):31-9. PMID: 12517701.
27. **Orth JD**, Krueger EW, Cao H, McNiven MA. From the cover: The large GTPase dynamin regulates actin comet formation and movement in living cells. *Proc Natl Acad Sci U S A*. 2002 Jan 8;99(1):167-72. PMC117533. Comments in: Editor's Choice in Cell Biology Highlight, "Dynamin and Propulsive Comet Tails", *Science*, 295, p587, 2002; Highlights, Cytoskeleton, "Comet tales", *Nat Rev Mol Cell Biol*, 3, p81, 2002; Paper alert, cell biology, cell structure and dynamics, *Curr Opin Cell Biol*, 14, p127, 2002.
28. Hart PE, Poynter GM, Whitehead CM, **Orth JD**, Glantz JN, Busby RC, Barrett SL, Salisbury JL. Characterization of the X-linked murine centrin Cetn2 gene. *Gene*. 2001 Feb 21;264(2):205-13. PMID: 11250075.
29. McNiven MA, Kim L, Krueger EW, **Orth JD**, Cao H, Wong TW. Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape. *J Cell Biol*. 2000 Oct 2;151(1):187-98. PMC2189798.
30. Hart PE, Glantz JN, **Orth JD**, Poynter GM, Salisbury JL. Testis-specific murine centrin, Cetn1: genomic characterization and evidence for retroposition of a gene encoding a centrosome protein. *Genomics*. 1999 Sep 1;60(2):111-20. PMID: 10486202.

Book chapter:
31. **Orth JD**, Gray NW, Thompson HM, McNiven MA. Dynamin and Cytoskeletal-dependent Membrane Processes, p 189-99; Cell Motility: From Molecules To Organisms, *John Wiley & Sons, Ltd*, 2004.

E. Research Support

Active Research Support

University of Colorado Boulder
Start-up Fund Package (James Orth) 09/2012 – present
Title: "Defining Molecular Mechanisms of Anti-cancer Therapeutics"
Total Award Amount: $350,000
Goals: To establish an active research group consisting of graduate students, post-doctoral fellows and/or professional research associates focused on understanding anti-cancer drug action at the molecular, cellular, and tumor scale.
Role: PI

Karyopharm Therapeutics, Inc.
Research gift to University of Colorado Boulder (James Orth) 02/2013 - present
Title: "Drug mechanisms of novel anti-cancer therapeutics in solid cancer derived cells"
Total Award Amount: $30,000 (two independent gifts of $15,000 were awarded)
Goals: Academic cancer research with investigational lead compounds to gain insight into molecular mechanism. Compounds are targeted against specific proteins and enzymes involved in various cancers. Research is directed solely and independently by the Orth laboratory. Dr. Orth is not compensated with salary, stock, or in any other means by Karyopharm Therapeutics, Inc.
Role: PI

Completed Research Support

American Cancer Society (University of Colorado Cancer Center)

ACS IRG #57-001-53 (James Orth) 01/2015 – 12/2017
Title: “Targeting Nuclear Export for Pancreatic Cancer Therapy”
Total Award Amount: $30,000
Goals: The major goal of this seed grant is to generate cell line tools like those in the current proposal, and to identify the major cell responses to nuclear export inhibition. We plan to translate the work to clinical relevance via intravital microscopy of xenograft and patient derived xenograft models through collaborators at the University of Colorado Cancer Center or Massachusetts General Hospital (Ralph Weissleder).
Role: PI
Co-PIs: -

Research Grants in Preparation

Source of Support: Roche, collaborative research mechanism
Title: “Multi-pronged ‘omics methods to identity new agents that selectively target and kill quiescent and therapy-induced senescent cancer cells”
Total Award Amount: TBD
Goals: To identify novel small molecules that kill quiescent cells in tumors and senescent cancer cells that have survived chemotherapy and define the mechanisms of synthetic lethality.
Total Award Period Covered: TBD
Location of Project: University of Colorado Boulder
Person-Months per Year Committed to Project:
Status: telephone interview with liaison completed, research project idea submission form submitted
Role: PI
Co-PIs: -

Source of Support: NCI, RO1, other. Plan to submit for June 2019
Title: “Linking nuclear envelope rupture to the proliferation rate paradox during Taxane response”
Total Award Amount: $1,250,000
Goals: To define mechanisms of nuclear envelope rupture in cells treated with Taxol from *in vitro* models to human, and progress toward an understanding of the role of rupture during tumor response and rupture as a possible predictor of response, trigger of inflammation and large-scale tumor loss particularly in sarcomas and triple negative breast cancers.
Total Award Period Covered: TBD
Location of Project: University of Colorado Boulder and Harvard Medical School
Person-Months per Year Committed to Project: 6
Status: in preparation
Role: PI
Co-PIs: none

Source of Support: Appropriate for multiple sources, e.g. R21 PA-17-4439 “The Interplay of Cell Death Pathways in Cancer Cell Survival and Resistance to Therapy”; Sarcoma Foundation of America, others.
Title: “Phenomics, adaptive drug resistance, and TP53: Considerations for targeting nuclear export in cancers”.
Total Award Amount: TBD
Goals: To understand adaptive drug mechanism for exportin-1 inhibitors, how this alters phenotypic response to exportin-1 inhibitors and other cancer drugs, and how modulation of tumor suppressor signaling via p53 impacts resistance. Novel drug combinations will be defined based on mechanistic findings of drug resistance.
Location of Project: University of Colorado Boulder
Person-Months per Year Committed to Project: TBD
Status: draft in revision
Role: PI
Co-PIs: -

Source of Support: Appropriate for multiple sources; NCI, Sarcoma Foundation of America, CLC, others
Title: “Targeting therapy-induced senescent cells to improve anti-cancer drug action”
Total Award Amount: TBD
Goals: To identify small molecules that selectively kill senescent cells remaining after small molecule chemotherapeutic treatment and characterize their mode of action

Location of Project: University of Colorado Boulder
Person-Months per Year Committed to Project: TBD
Status: draft in revision
Role: PI
Co-PIs: -

Equipment Grants Awarded:

Source of Support: NSF (MRI)
Title: “Collaborative Research: MRI Consortium: Development of Fiber-coupled stimulated emission depletion microscopy (STED)”
Total Award Amount: equipment development grant
Goals: To build a fiber-mounted stimulated emission depletion (STED) super resolution microscope that can be mounted into a sample (live animal or isolated tissue) to perform intravital microscopy
Total Award Period Covered: n/a
Location of Project: University of Colorado Boulder
Person-Months per Year Committed to Project: TBD
Status: Awarded, 8/2019
Role: collaborator and management of instrument
Co-PIs: Major PI is Juliet Gopinath (ECEE)

Equipment Grants Submitted:

Source of Support: IR-IST
Title: “A transformative approach to *biological* microscopy imaging that links breakthroughs in nanoparticle contrast agent development to innovations in computational optical super-resolution imaging”
Total Award Amount: equipment development grant
Goals: Implement novel sub-diffraction limited super resolution microscopy technology, and engineer improved upconversion nanoparticles (UCNPs) as improved signal-to-noise agents for biological super-resolution microscopy to enable chemically specific labelling of cell components in studies of cell function.
Total Award Period Covered: n/a
Location of Project: University of Colorado Boulder
Person-Months per Year Committed to Project: TBD
Status: Submitted 1/2019
Role: Collaborator. Advice on instrument modification and management. Evaluation and implementation of new technology.
Co-PIs: PI Carol Cogswell (ECEE); Co-PIs Won Park (ECEE) and Stephen Becker (Applied Math)