TITLE:	**Targeting 6-Phosphofructo-2-Kinase To Increase the Efficacy of ER and CDK4/6 Inhibitors Against Metastatic Breast Cancer**
Principal Investigator:	Yoannis Imbert-Fernandez, Ph.D
Applicant Organization:	University of Louisville Research Foundation
Address:	505 South Hancock Street Room 427A Clinical and Translational Research Building Louisville, KY 40202
Phone:	502-852-3686
Email:	Yoannis.imbertfernandez@louisville.edu

PATHWAY TO "RO1" STATEMENT

This application seeks to address the challenge to eliminate the mortality associated with breast cancer by examining the potential for PFKFB3 inhibitors to increase the anti-breast cancer efficacy of two small molecules, fulvestrant and palbociclib, that are FDA-approved for the treatment of stage IV breast cancer. The results of this study are expected to provide the needed rationale to expand our initial observations into an RO1 proposal. The application will be written to include the following aims:

1. **To determine the effects of simultaneous PFKFB3 and CDK4/6 inhibition on the utilization of glucose and glutamine using ^{13}C-isotopomer metabolomics analyses by NMR and mass spectrometry.** *Rationale:* Active Rb suppresses glucose and glutamine metabolism and we postulate that dual targeting of PFKFB3 (and thus CDK1) and CDK4/6 inhibition will cause a marked suppression of metabolic flux via reduced Rb-phosphorylation and increased Rb function, especially in the absence of estrogen or presence of fulvestrant. We will propose to analyze glucose and glutamine utilization pathways using NMR and mass spectrometry metabolomics approaches that we have established in our lab after PFKFB3 and CDK4/6 inhibition.

2. **Examination of the roles of PFKFB3 and CDK4/6 in endocrine-resistant breast cancer cells.** *Rationale:* 25 to 50% of newly diagnosed ER+ breast cancer patients will not respond at all to anti-estrogen therapies and, importantly, most patients that initially responded to these agents eventually become resistant during the course of therapy (acquired resistance). Interestingly, the E2-induced metabolic flare on FDG-PET scans is predictive of breast cancer endocrine responsiveness highlighting the importance of glucose metabolism in E2-driven breast cancer tumors. Given that PFKFB3 and CDK4/6 are targets downstream of endocrine therapy we will postulate that combining PFK158 with palbociclib and/or fulvestrant in endocrine resistant cells will cause a synergistic increase in cell death and re-sensitize ET resistant cells to endocrine therapies

3. **To assess the capacity of the PFKFB3 inhibitor, PFK158, to prolong the anti-tumor activity of fulvestrant and palbociblib against ER+ patient-derived xenograft (PDX) models and to prolong the anti-tumor activity of palbociclib against three TN PDX models in NGS mice.** *Rationale:* A limitation of the *in vivo* xenograft models generated by implanting cultured cell lines that we have proposed in aim 2 is their inadequate predictive value for future outcomes. Given that our long-term objective is to generate sufficient pre-clinical data for a phase 1/2 clinical trial in stage IV ER+ and TN breast cancer patients, we will propose to study the potential utility of PFK158 in the more relevant PDX models.

These preliminary studies will provide enough rationale to submit an RO1 in the fall of 2016 and will markedly improve the potential of the application to get funded, which will have a major impact on breast cancer patients.

BUDGET JUSTIFICATION

Personnel

Yoannis Imbert-Fernandez, Ph.D., will function as the P.I. and no salary is requested annually. Dr. Imbert-Fernandez demonstrated that estrogens stimulate PFKFB3 which was found to be essential for breast cancer cell glucose metabolism and survival. Dr. Imbert-Fernandez is currently funded through the DOD CDMRP Breast Cancer Post-Doctoral Fellowship to study the regulation of glucose utilization by estradiol in breast cancer.

Amy Clem, M.S., will serve as the laboratory technician and 4 months of her salary is requested annually. She will conduct the combination studies proposed in Specific Aim 1 using siRNA and small molecular inhibitors and will interrogate the novel hypothesis that PFK158 may be able to disrupt the CDK1 compensation that occurs after exposure to palbociclib.

A 3% inflation increment has been factored after Year 1 for cost-of-living salary increases. Fringe benefits are charged at their actual rate which is currently 37.67%.

Budget

The following categories are requested

BUDGET CATEGORY		INITIAL BUDGET PERIOD (YEAR 1)	INITIAL BUDGET PERIOD (YEAR 2)
SALARY		17,084	17,596
MATERIALS AND SUPPLIES		78,154	77,642
TRAVEL COST		0	0
INDIRECT COSTS		4,762	4,762
TOTAL COSTS		100,000	100,000
TOTAL COSTS FOR ENTIRE PROPOSED PERIOD		**100,000**	**100,000**

The University of Louisville's Department of Purchasing has policies and procedures in place for goods and services. The policies are located at http://louisville.edu/purchasing/policies.

Six-Month Progress

The PI will provide a progress report six months after the initiation of the project. The progress will include an updated budget and the milestones achieved during the period funded.

PUBLIC ABSTRACT: **Targeting 6-Phosphofructo-2-Kinase To Increase the Efficacy of ER and CDK4/6 Inhibitors Against Metastatic Breast Cancer**

Breast tumors are driven to grow through a combination of increased breast cancer cell sugar metabolism and proliferation. An enzyme called 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) regulates both of these two biological processes by activating two key downstream enzymes, 6-phosphofructo-2-kinase (PFK-1) and cyclin-dependent kinase 1 (CDK1). Estrogens stimulate PFKFB3 which was found to be essential for breast cancer cell glucose metabolism and survival in 2014. This year, a drug, palbociclib, that inhibits other estrogen-regulated enzymes, cyclin-dependent kinases 4 and 6, was found to improve the survival of breast cancer patients when combined with anti-estrogen agents. We postulated that simultaneous inhibition of both of these estrogen-regulated enzymes (PFKFB3 and CDK4/6) would be catastrophic to breast cancer cells but not to normal cells. In new, unpublished studies, we have found that a PFKFB3 inhibitor (called PFK158) not only improves the anti-breast cancer activity of the widely used anti-estrogen drug, fulvestrant, in mice but also increases the anti-breast cancer activity of palbociclib *in vitro*. Based on these studies, we believe that PFK158 will increase the activity of anti-estrogen (*e.g* fulvestrant) and anti-CDK4/6 (*e.g.* palbociclib) drugs to durably shrink tumors and hopefully improve the survival of estrogen receptor (ER)+ breast cancer patients. We also believe that PFK158 may improve the activity of anti-CDK4/6 inhibitors in the absence of anti-estrogen agents in triple-negative (TN) breast cancer patients. Importantly, we developed PFK158 at the University of Louisville and are conducting a phase 1 trial that includes breast cancer patients at four institutions including the University of Louisville, MD Anderson Cancer Center, UT Southwestern and Georgetown University. Accordingly, we are confident that if we find that PFK158 improves the activity of these FDA-approved drugs in our highly relevant mouse models of breast cancer, then we will be able to quickly initiate multiple phase 1/2 trials to test these combinations in women suffering from both ER+ and TN breast cancer.

OVERARCHING CHALLENGE: This application seeks to address the challenge to eliminate the mortality associated with breast cancer by examining the potential for PFKFB3 inhibitors to increase the anti-breast cancer efficacy of two small molecules, fulvestrant and palbociclib, that are FDA-approved for the treatment of stage IV breast cancer.

HYPOTHESIS: We postulate that PFKFB3 inhibitors may be able to increase the efficacy of ER antagonists and CDK4/6 inhibitors through combined suppression of glucose metabolism and CDK1 activity.

SPECIFIC AIMS:

1. To determine the effects of combined ER, CDK4/6 and PFKFB3 inhibition on glucose metabolism, cell cycle regulators, growth and survival *in vitro*.
2. To examine the anti-metabolic and anti-growth effects of fulvestrant, palbociclib and PFK158 as monotherapies and in combination in mouse models of breast cancer *in vivo*.

CLINICAL APPLICATION: The results of this study may provide the rationale to conduct phase 1/2 clinical trials of PFK158 in combination with anti-estrogen agents and CDK4/6 inhibitors in ER+ breast cancer patients and in combination with CDK4/6 inhibitors in TN breast cancer patients. If we observe clinical responses in these early phase trials, then we intend to conduct phase 3 trials to determine the potential of PFK158 to improve the survival of breast cancer patients. Although the potential benefits are clear, the risks are not as we have not observed any serious adverse events related to PFK158 in the phase 1 clinical trial (expected completion is in early 2016). The proposed pre-clinical studies should take 3 years but, if we observe data consistent with improved outcomes during the first 1-2 years of this grant, then we would develop the phase 1/2 clinical trial protocols in order to initiate these trials before the end of the three-year grant period.

POTENTIAL IMPACT: Our proposed studies to test the hypothesis that PFK158 may synergistically increase the activity of palbociclib with and without fulvestrant in pre-clinical models is anticipated to provide the rationale to conduct IND-enabling toxicity studies and phase 1/2 trials of these combinations in TN and ER+ stage IV breast cancer which in turn is expected to result in improved clinical outcomes for patients suffering from metastatic breast cancer.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Imbert-Fernandez, Yoannis**

eRA COMMONS USER NAME (credential, e.g., agency login): **yimire01**

POSITION TITLE: **Postdoctoral Scholar**

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Havana, Cuba	**B.A.**	**1998**	**Biochemistry**
University of Louisville, Louisville, KY	**M.S.**	**2008**	**Biochemistry**
University of Louisville, Louisville, KY	**Ph.D**	**2010**	**Biochemistry**
James Graham Brown Cancer Center, Louisville, KY	**Post-Doc**	**2010**	**Medicine (Medical Oncology/Hematology)**

A. Positions and Honors

Positions and Employment

1998 – 2000 Associate Researcher, National Coordinatinf Center of Clinical Trials. Havana, Cuba (Supervisor: Grisel Gonzalez)

2002 – 2003 Volunteer, Department of Molecular and Craniofacial Biology, University of Louisville, Louisville, KY (Supervisor: William W. Young, Ph.D.)

2003 – 2005 Laboratory technician, Department of Molecular and Craniofacial Biology, University of Louisville, Louisville, KY (Supervisor: William W. Young, Ph.D.)

2011 – Present Post-Doctoral Scholar, Division of Hematology/Oncology, Department of Medicine, University of Louisville, KY (Mentor: Jason Chesney, M.D., Ph.D.)

Honors

2007-2010 NIH Pre-Doctoral Fellowship, The National Eye Institute
2010 Graduate Dean's Citation, University of Louisville
2012 Invited speaker at the Molecular Targets Group at the James Graham Brown Cancer Center. University of Louisville. Title: Estradiol: How sweet it is.
2012 Invited speaker at the Department of Biochemistry and Molecular Biology, University of Louisville. Title: Fructose-2,6-Bisphosphate- An Essential Effector Molecule of Estradiol-Induced Glucose Metabolism and Growth.
2012 Ralph Scott Fellow Basic Research Prize 3rd place (JGBCC Retreat)

2013 Ralph Scott Fellow Basic Research Prize 1st place (JGBCC Retreat)
2014 Ralph Scott Fellow Basic Research Prize 2nd place (JGBCC Retreat)

B. Peer-reviewed publications *(Yoannis Imbert became Yoannis Imbert-Fernandez in 2010)*

1. **Imbert, Y.**, Darling, D.S., Jumblatt, M.M., Foulks, G.N., Couzin, E.G., Steele, P.S., and Young, W.W., Jr. MUC1 splice variants in human ocular surface tissues: possible differences between dry eye patients and normal controls. Exp. Eye Res. 2006 Sep;83(3):493-501. Epub 2006 Apr 21. PMID: 16631167

2. Jumblatt, M.M., **Imbert, Y.**, Young, W.W. Jr., Foulks, G.N., Steele, P.S., and Demuth, D.R. Glycoprotein 340 in normal human ocular surface tissues and tear film. Infect Immun. 2006 Jul;74(7):4058-63. PMID: 16790779

3. **Imbert, Y.**, Jumblatt, M.M., Foulks, G.N., Couzin, E.G., Steele, P.S., Young, W.W. Jr. Expression in human ocular surface tissues of the GalNAc-transferases that initiate mucin-type O-glycosylation. Cornea. 2006 Dec;25(10):1193-9. PMID: 17172897

4. **Imbert, Y.**, Foulks, G.N., Brennan, M.D., Jumblatt, M.M., John, G., Shah, H.A., Newton, C., Pouranfar, F., Young, W.W., Jr. MUC1 and estrogen receptor alpha gene polymorphisms in dry eye patients. Exp Eye Res. 2009 Mar;88(3):334-8. Epub 2008 Jun 20. PMID: 18619437

5. Schultz, D.J., Wickramasinghe, N.S., Ivanova, M.M., Isaacs, S.M., Dougherty, S.M., **Imbert-Fernandez, Y.**, Cunningham, A.R., Chen, C., Klinge, C.M. Anacardic acid inhibits estrogen receptor alpha-DNA binding and reduces target gene transcription and breast cancer cell proliferation. Mol Cancer Ther. 2010 Mar;9(3):594-605. Epub 2010 Mar 2. PMID: 20197399

6. **Imbert-Fernandez, Y.**, Radde, B.N., Teng, Y., Young, W.W., Jr., Hu, C., Klinge, C.M. MUC1/A and MUC1/B splice variants differentially regulate inflammatory cytokine expression. Exp Eye Res. 2011 Nov;10(11):2062-71. Epub 2011 Aug 16. PMID: 21862684

7. Klinge, C.M., Radde, B.N., **Imbert-Fernandez, Y.**, Teng, Y., Ivanova, M.M., Abner, S.M. and Martin, A.L. Targeting the intracellular MUC1 C-terminal domain inhibits proliferation and estrogen receptor transcriptional activity in lung adenocarcinoma cells. Mol Cancer Ther. 2011 Nov;93(5):649-57. Epub 2011 Aug 23. PMID: 21854773

8. Telang, S., Nelson, K.K., Siow, D.L., Yalcin, A., Thornburg, J.M., **Imbert-Fernandez, Y.**, Klarer, A.C., Farghaly, H., Clem, B.F., Eaton, J.W., Chesney, J. Cytochrome c oxidase is activated by the oncoprotein Ras and is required for A549 lung adenocarcinoma growth. Mol Cancer. 2012 Aug 23;11(1):60. Epub ahead of print. PMID: 22917272

9. Clem, B.F, O'Neal, J., Tapolsky, G., Clem, A.L., **Imbert-Fernandez, Y.**, Kerr, D.A. 2nd, Klarer, A.C, Redman, R., Miller, D.M., Trent, J.O., Telang, S., Chesney, J. Targeting 6-Phosphofructo-2-Kinase (PFKFB3) as a Therapeutic Strategy against Cancer. Mol Cancer Ther. 2013 Aug;12(8):1461-70.. Epub 2013 May 14.

10. Klarer, A.C., O'Neal, J., **Imbert-Fernandez, Y.**, Clem, A., Ellis, S.R., Clark, J., Clem, B., Chesney, J., Telang, S. Inhibition of 6-phosphofructo-2-kinase (PFKFB3) induces autophagy as a survival mechanism. Cancer Metab. 2014 Jan 23;2(1):2. PMID: 24451478

11. **Imbert-Fernandez, Y.**, Clem, B.F., O'Neal, J., Kerr, D.A., Spaulding, R., Lanceta, L., Clem, A.L., Telang, S., Chesney J. Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3). JBC. 2014 Mar 28;289(13)9440-8. PMID:24515104

12. Yalcin, A., Clem, B.F., **Imbert-Fernandez, Y.**, Ozcan, S.C., Peker, S., O'Neal, J., Klarer, A.C, Clem, A.L., Telang, S., Chesney, J. 6-phosphofructo-2-kinase (PFKFB3) promotes cell cycle progression and suppresses apoptosis via Cdk1-mediated phosphorylatin of p27. Cell Death Disease. 2014 July 17. PMID: 25032860

13. Chesney, J., Clark, J., Klarer, A.C., **Imbert-Fernandez, Y.**, Lane, A.N., Telang, S. Fructose-2,6-bisphosphate synthesis by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is required for the glyclolytic response to hypoxia and tumor growth. Oncotarget. 2014 July 13 PMID:25115398. Epub ahead of print.

Poster Presentations

1. **Imbert, Y.**, Darling, D.S., Jumblatt, M.M., Foulks, G.N., Couzin, E.G., Steele, P.S. and Young, W.W., Jr. Mucin splice variants in ocular surface tissues. (Abstract 75). Glycobiology 15, 1220, 2005.

2. **Imbert, Y.**, Darling, D.S., Jumblatt, M.M., Foulks, G.N., Couzin, E.G., Steele, P.S. and Young, W.W., Jr. Mucin splice variants in the human ocular surface: possible differences between dry eye patients and normal controls. (Abstract GRD24) Research! Louisville, 2005.

3. **Imbert, Y.**, Darling, D.S., Jumblatt, M.M., Foulks, G.N., Couzin, E.G., Steele, P.S. and Young, W.W., Jr. Mucin splice variants in the human ocular surface: possible differences between dry eye patients and normal controls. (Abstract 5596). ARVO meeting, IOVS, 2006.

4. **Imbert, Y.**, G.N. Foulks, M.D. Brennan, M.M. Jumblatt, G. John, H.A. Shah, C. Newton, and W.W. Young, Jr. MUC1 gene polymorphism in dry eye patients. (Abstract 6). TFOS meeting, 2007.

5. **Imbert-Fernandez, Y.**, and Klinge, C.M. MUC1 splice variants differentially regulates inflammatory responses in transfected COS-7 cells. (Abstract 753). FASEB meeting, 2010.

6. Radde, B.N., **Imbert-Fernandez, Y.** and Klinge, C.M. MUC1-Estrogen Receptor interaction in lung adenocarcinoma cells (Abstract RS-104). Research!Louisville, 2010.

7. **Imbert-Fernandez Y.**, Clem, B., O'Neal, J., Clem, A. and Chesney, J. Estradiol stimulates 6-phosphofructo-2-kinase (PFKFB3) expression and glycolysis by breast cancer cells (Abstract PRF-45). Research!Louisville, 2011.

8. Spaulding, R., **Imbert-Fernandez, Y.**, Telang, S., Clem, B.F., Trent, J.O., Chesney, J. Discovery of a novel small molecule antagonist of cytosolic aspartate aminotransferase that causes decreased transformed cell growth in vitro (Abstract MED-82). Research!Louisville, 2012.

9. **Imbert-Fernandez Y.**, Clem, B., O'Neal, J., Clem, A. and Chesney, J. Stimulation of glucose metabolism by estradiol is mediated by 6-phosphofructo-2-kinase (PFKFB3) (Abstract 51). 11th Annual Retreat, Brown cancer Research, 2012.
10. **Imbert-Fernandez Y.**, Clem, B., O'Neal, J., Clem, A. and Chesney, J. Stimulation of glucose metabolism by estradiol is mediated by 6-phosphofructo-2-kinase (PFKFB3) (Abstract X4 2011). Tumor metabolism meeting. Keystone symposia, 2013.

11. **Imbert-Fernandez Y.**, Clem, B., O'Neal, J., Clem, A. and Chesney, J. Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3). 12th Annual Retreat, Brown cancer Research, 2013.

12. **Imbert-Fernandez Y.**, Clem, B., O'Neal, J., Clem, A. and Chesney, J. Simultaneous inhibition of the estrogen rece and 6-phosphofructo-2-kinase (PFKFB3) for the treatment of ER+ breast cance (Abstract P29) Metabolism, diet and disease, 2014.

13. **Imbert-Fernandez Y.**, Clem, B., Clem, A. and Chesney, J. Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3) (Abstract 40). 13th Annual Retreat, Brown cancer Research, 2014.

C. Current Support

1. DOD CDMRP Breast Cancer Post-Doctoral Fellowship (Imbert-Fernandez)

Title:	**Regulation of Glucose Utilization by Estradiol In Breast Cancer**
Role:	*Principal Investigator (100%)*
Period of Support:	7/01/2013-6/30/2016
Total Award:	$447,226

This grant proposal is to fund my training to become a breast cancer researcher.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **Clem, Amy**

eRA COMMONS USER NAME (credential, e.g., agency login): **alclem01**

POSITION TITLE: **Research Technologist Senior**

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Montevallo, Alabama	**B.S.**	**1999**	**Biology**
University of Louisville, Louisville, KY	**M.S.**	**2003**	**Biochemistry**

A. Positions and Honors

Positions and Employment

2000 – 2001 Post-baccalaureate program, National Institutes of Health, Bethesda

2001 – 2003 Research technologist II, University of Louisville, Louisville, KY (Supervisor: Sham Kakar, Ph.D.)

2003 – 2006 Research technologist II, University of Louisville, Louisville, KY (Supervisor: Jason Chesney, M.D, Ph.D.)

2006 – Present Research technologist senior, University of Louisville, Louisville, KY (Supervisor: Jason Chesney, M.D, Ph.D.)

B. Peer-reviewed publications

1. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. Devin A, **Cook A**, Lin Y, Rodriguez Y, Kelliher M, Liu Z. Immunity. 2000 Apr;12(4):419-29.
2. The death domain kinase RIP is essential for TRAIL (Apo2L)-induced activation of IkappaB kinase and c-Jun N-terminal kinase. Lin Y, Devin A, **Cook A**, Keane MM, Kelliher M, Lipkowitz S, Liu ZG. Mol Cell Biol. 2000 Sep;20(18):6638-45.
3. Characterization of the role of Sp1 and NF-Y in differential regulation of PTTG/securin expression in tumor cells. **Clem AL**, Hamid T, Kakar SS. Gene. 2003 Dec 11; 322:113-21.
4. Targeted disruption of inducible 6-phosphofructo-2-kinase results in embryonic lethality. Jason Chesney, Sucheta Telang, Abdullah Yalcin, **Amy Clem**, Natalie Wallis and Richard Bucala. Biochem Biophys Res Commun. 2005 May 27;331(1):139-46.
5. Ras transformation requires metabolic control by 6-phosphofructo-2-kinase.Telang S, Yalcin A, **Clem AL**, Bucala R, Lane AN, Eaton JW, Chesney J. Oncogene. 2006 Nov 23;25(55):7225-34.

6. Depletion of ascorbic acid restricts angiogenesis and retards tumor growth in a mouse model. Telang S, **Clem AL**, Eaton JW, Chesney J. Neoplasia. 2007 Jan;9(1):47-56.

7. Virus detection and identification using random multiplex (RT)-PCR with 3'-locked random primers. **Clem AL**, Sims J, Telang S, Eaton JW, Chesney J. Virol J. 2007 Jun 28;4:65.

8. Transient T cell depletion causes regression of melanoma metastases. Rasku MA, **Clem AL**, Telang S, Taft B, Gettings K, Gragg H, Cramer D, Lear SC, McMasters KM, Miller DM, Chesney J. Journal of Translational Medicine 2008 Mar 11;6:12.

9. Small molecule inhibition of 6-phosphofructo-2-kinase activity suppresses Glycolytic flux and tumor growth. Clem B, Telang S, **Clem A**, Yalcin A, Meier J, Simmons A, Rasku MA, Arumugam S, Dean WL, Eaton J, Lane A, Trent JO, Chesney J. Mol Cancer Ther 2008;7(1)110-20.

10. Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, **Clem AL**, Brock E, Siow D, Wattenberg B, Telang S, Chesney J. J Biol Chem. 2009 Sep 4;284(36):24223-32. Epub 2009 May 27.

11. Selective inhibition of choline kinase simultaneously attenuates MAPK and PI3K/AKT signaling. Yalcin A, Clem B, Makoni S, **Clem A**, Nelson K, Thornburg J, Siow D, Lane AN, Brock SE, Goswami U, Eaton JW, Telang S, Chesney J. Oncogene. 2010 Jan 7;29(1):139-49. Epub 2009 Oct 26.

12. A Novel Small Molecule Antagonist of Choline Kinase-α That Simultaneously Suppresses MAPK and PI3K/AKT Signaling. Clem B, **Clem A**, Yalcin A, Goswami U, Arumugam S, Telang S, Trent JO, Chesney J. Oncogene.

13. Phase II trial of the regulatory T cell-depleting agent, denileukin diftitox, in patients with unresectable stage IV melanoma. Telang S, Rasku MA, **Clem AL**, Carter K, Klarer AC, Badger WR, Milam RA, Rai SN, Pan J, Gragg H, Clem BF, McMasters KM, Miller DM, Chesney J.BMC Cancer. 2011 Dec 13;11:515. doi: 10.1186/1471-2407-11-515.

14. Small molecule inhibition of 6-phosphofructo-2-kinase suppresses t cell activation. Telang S, Clem BF, Klarer AC, **Clem AL**, Trent JO, Bucala R, Chesney J.J Transl Med. 2012 May 16;10:95. doi: 10.1186/1479-5876-10-95.

15. Targeting 6-phosphofructo-2-kinase (PFKFB3) as a therapeutic strategy against cancer. Clem BF, O'Neal J, Tapolsky G, **Clem AL**, Imbert-Fernandez Y, Kerr DA 2nd, Klarer AC, Redman R, Miller DM, Trent JO, Telang S, Chesney J.Mol Cancer Ther. 2013 Aug;12(8):1461-70. doi: 10.1158/1535-7163.MCT-13-0097. Epub 2013 May 14.

16. Inhibition of 6-phosphofructo-2-kinase (PFKFB3) induces autophagy as a survival mechanism. Klarer AC, O'Neal J, Imbert-Fernandez Y, **Clem A**, Ellis SR, Clark J, Clem B, Chesney J, Telang S.Cancer Metab. 2014 Jan 23;2(1):2. doi: 10.1186/2049-3002-2-2.

17. Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3).Imbert-Fernandez Y, Clem BF, O'Neal J, Kerr DA, Spaulding R, Lanceta L, **Clem AL**, Telang S, Chesney J.J Biol Chem. 2014 Mar 28;289(13):9440-8. doi: 10.1074/jbc.M113.529990. Epub 2014 Feb 10.