Activation of Akt pathway and autophagy promotes resistance to FASN inhibition in colorectal cancer patient-derived xenograft models

Yekaterina Y. Zaytseva, Piotr G. Rychahou, Anh-Thu Le, Robert M. Flight, Timothy L. Scott, Jennifer Harris, Sally Hodges, Brent J. Hallahan, Dana L. Napier, Jinpeng Liu, Chi Wang, Manjula Sunkara, Andrew Morris, Ji Tae Kim, Sivakumaran Theru Arumugam, Andrew Lane, Teresa Fan, Hunter Moseley, Tianyan Gao, Eun Y. Lee, Heidi L. Weiss, Timothy S. Heuer, George Kemble, and B. Mark Evers

Department of Toxicology and Cancer Biology, Department of Internal Medicine, and Markey Cancer Center, University of Kentucky, Lexington; 3-V Biosciences, Menlo Park, California

Background

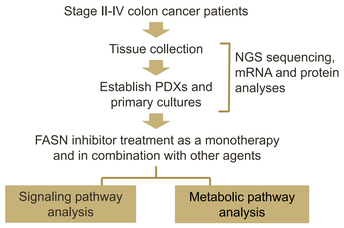
Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States. Fatty acid synthase (FASN), a key enzyme of lipid biogenesis, is significantly up-regulated and activated in many cancers including CRC and its activity is associated with poor prognosis, higher risk of disease recurrence, and death. **What we know about colorectal cancer and *de novo* lipogenesis**:

- Increased expression and activity of FASN is associated with enhanced cellular proliferation and metastasis in CRC;
- Oral FASN inhibitor (TVB-2640) entered a Phase I clinical trial (3V2640-CLIN-002) in solid tumor patients demonstrating a favorable tolerability profile with no significant adverse events (3V-Biosciences);
- Tumor characteristics that would indicate responsiveness to FASN inhibition are not fully understood.

Purpose

(i) to determine the effect of FASN inhibition on tumor growth in CRC patient-derived xenografts (PDXs);
(ii) to identify potential biomarkers associated with CRC responsiveness to FASN inhibition;
(iii) to explore new combination strategies with FASN inhibitors.

Study Design



Materials & Methods

- **Human tissues analysis:** Human CRC and matching normal colonic tissues were obtained from surgical patients at UK Chandler Hospital. Tissue microarray was developed by Dr. Eun Lee and his colleagues in the Markey Cancer Center Biospecimen Core.
- ***In vivo* studies**: The effect of TVB-3664 on tumor growth was assessed in PDX models established in NOD SCID gamma mice using freshly resected CRC specimens (either primary CRC or metastasis) from consented surgical patients at UK Chandler Hospital at the time of operation (IRB#13-0753-P2H). Once the xenografts grew to ~100 mm^3, mice were randomized into two groups (n=5) to receive either vehicle or TVB-3664 by gavage daily. Tumor volume and animal weights were measured weekly. Tumor volume was expressed in mm^3 using the formula: $V = width^2 \times length \times 0.5$. The study was terminated 4 h after the final dose. Western blot analysis and immunohistochemistry staining were used to identify FASN-mediated changes in major oncogenic pathways. Blood samples were collected from control and treated groups prior the sacrifice and the level of palmitate was determined in plasma by mass spectrometry.
- **Statistical analysis:** Paired tests using the Wilcoxon signed rank test were employed to assess differential expression of FASN immunoreactivity scores between matched normal versus tumor tissues. Tumor volume was analyzed using linear mixed models for each patient PDX model. Since there is no clear trend in tumor volume over time, an overall comparison of trend was not employed. Instead, a linear mixed model with fixed effects for treatments, time and their interaction and a random effect for the intercept term was employed in order to perform individual comparisons between groups at each time point.

Results

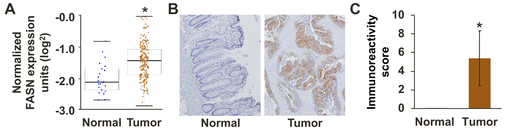
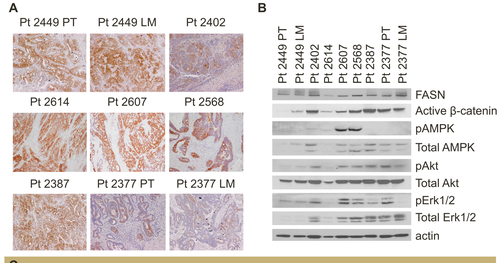


Figure 1. FASN expression is significantly upregulated in colon cancer tissues. (A) FASN mRNA expression is increased in CRC patient samples in the TCGA dataset (n=22 normal and 215 tumor samples, p<0.0001). **(B)** Representative IHC images from pair-matched CRC tissues stained with FASN antibody and counter stained with hematoxylin (TMA ID BH15991A). **(C)** Immunoreactivity score of FASN expression was analyzed in matched normal colon mucosa and tumor tissues from patients who were diagnosed with Stage I-IV CRC and had surgery at UK Chandler Medical Center (n=57 normal and 56 tumor tissues, *p<0.001 as compared to normal tissue). **(D)** Distribution of FASN immunoreactivity score in analyzed CRC tissues.

Table 1. PDX models used for evaluation of FASN inhibition. Demographic and clinical information on CRC patients whose tissues were used to establish PDXs and primary cell lines.

Specimen ID	Primary Staging	Age/ Gender	Pathology	PDX Generation
Pt 93*	T3N1bM1	63/M	Metastatic medullary cancer, morphologically consistent with metastasis from colon (peritoneum and abdominal wall)	Cells (g3)
Pt 130*	T3N0M1a	76/M	Metastatic colonic adenocarcinoma	Cells (g3)
Pt 2377	T3N0M1a	66/F	Metastatic colonic adenocarcinoma (primary tumor and liver metastases)	PTg1 / LMg1
Pt 2387	T3N0	49/F	Metastatic adenocarcinoma (lung) consistent with colorectal primary tumor	g2
Pt 2402	T3N1M1a	47/F	Metastatic adenocarcinoma (lung) consistent with colon primary tumor	g1
Pt 2449	T3N2M1a	61/F	Metastatic medullary colonic carcinoma (primary tumor and liver metastases)	PTg1 / LMg1
Pt 2568	T2N0	69/F	Moderately differentiated mucinous colonic adenocarcinoma	g1
Pt 2607	T4aN1a	65/M	Mucinous colonic adenocarcinoma	g1
Pt 2614	T2N0	72/M	Moderately differentiated colonic adenocarcinoma	g1



C

	APC	BRAF	KRAS	NRAS	PI3K	EGFR	FGFR	TP53
Pt 93*		mut	mut					
Pt 130*		mut	mut				mut	
Pt 2449 PT		mut						
Pt 2449 LM		mut						mut
Pt 2402								mut
Pt 2614	mut				mut			mut
Pt 2607		mut				mut		
Pt 2568	mut							mut
Pt 2387					mut			
Pt 2377 PT	mut		mut		mut			
Pt 2377 LM			mut		mut			

Figure 2. Expression of FASN and activation of FASN-associated oncogenic pathways in original tissues and PDX models used for evaluation of TVB-3664 as a monotherapy. (A) Representative IHC images of resected tumor tissues stained for FASN expression. **(B)** Western blot analysis of tissues from established PDX models (g1) showing expression / activation of major oncogenic pathways known to be regulated by *de novo* lipogenesis. **(C)** Mutational status of 198 cancer genes was assessed in tumor tissues obtained from CRC patients and in established PDX models and primary cells (*) by NSG sequencing. Mutational status of key oncogenes involved in CRC development and progression is shown.

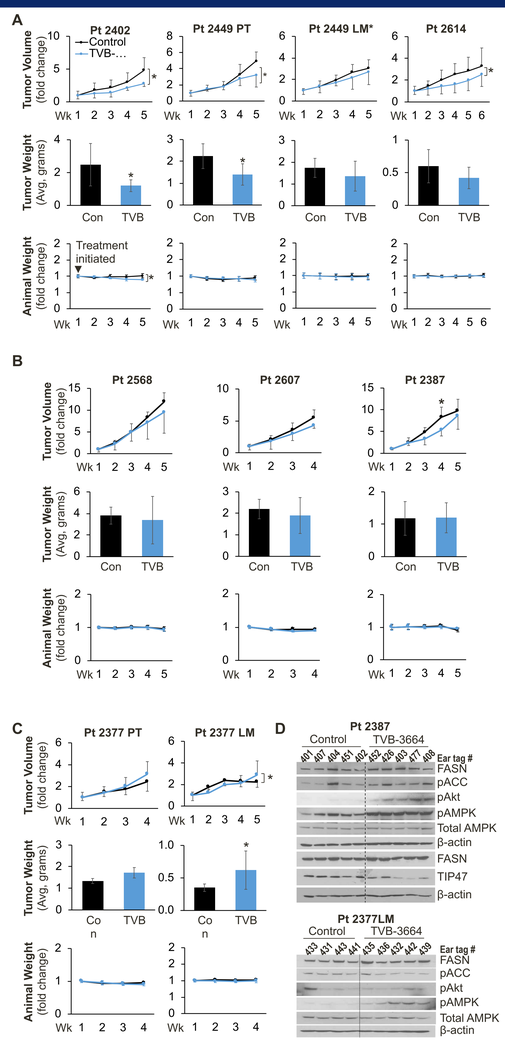
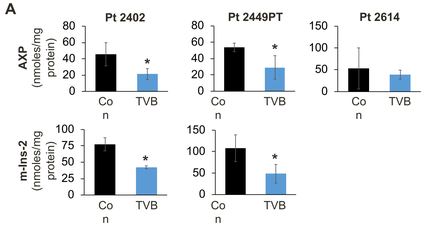
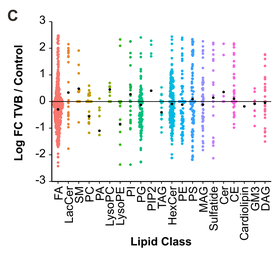


Figure 3. CRC PDX models exhibit a wide range of responses to FASN inhibition. (A) PDX models sensitive to TVB-3664 treatment. Tumor response to FASN inhibition is shown as a fold change in tumor volume over time. Bottom row shows corresponding fold change in weight of animals over time. Animals were treated daily with 3mg/kg (Pt 2614 and Pt 2449 PT) or 6 mg/kg (Pt 2402 and Pt 2449 LM) of TVB-3664 by oral gavage (*p<0.05). Pt 2377 PT and Pt 2377 LM are established from matched primary and liver metastasis tissues from Pt 2377. No response to TVB-3664 treatment was observed in PDX Pt 2449 LM. **(B)** PDX models resistant to FASN inhibition. Tumor response to FASN inhibition is shown as a fold change in tumor volume over time. Bottom row shows corresponding fold change in weight of animals over time. Animals were treated daily with 3mg/kg (Pt 2614 and Pt 2449 PT) or 6 mg/kg (Pt 2402 and Pt 2449 LM) of TVB-3664 by oral gavage (*p<0.05). **(C)** Accelerated tumor growth in Pt 2377 PDX models treated with 3mg/kg of TVB-3664. Tumor response to FASN inhibition is shown as a fold change in tumor volume over time in PDX models established from primary CRC (left) and liver metastasis tumors (right) Pt 2377. Bottom row shows corresponding fold change in weight of animals over time (*p<0.05). **(D)** Western blot analysis of tissues from PDX models which responded to FASN inhibition.



B	Control	TVB-3664	Control vs TVB		Control vs TVB	
Pt 2402	Mean	Mean	T-test p	T-test q	Wilcox p	Wilcox q
Glc.13C	731.215	3950.053	0.430	0.973	0.629	1.000
Total Glc	1259.261	3995.552	0.433	0.973	0.629	1.000
Lac13C	45.730	56.584	0.771	0.973	0.857	1.000
Total Lac	294.642	280.440	0.931	0.973	1.000	1.000
Pt 2449PT						
aGlc.13C	623.933	21123.755	0.150	0.131	0.016	0.033
Total Glc	1422.739	19340.908	0.145	0.131	0.016	0.033
Lac13C	210.333	143.446	0.654	0.211	0.730	0.615
Total Lac	718.715	461.852	0.465	0.211	0.905	0.693
Pt 2614						
Ace	145.288	192.142	0.028	0.375	0.016	0.265
Lac	2775.914	2426.603	0.308	0.375	0.548	0.479
Glc	7860.953	7411.248	0.646	0.375	0.690	0.479



D

Lipid Class	P-val	Log ratio 05	n up	n down
FA	0.000	-0.296	193	326
LacCer	0.003	0.336	42	18
SM	0.007	0.482	17	4
PC	0.017	-0.560	10	25
PA	0.039	-1.099	2	10
LysoPE	0.057	-0.847	3	11
LysoPC	0.057	0.452	11	3
PI	0.110	0.272	21	11
PG	0.140	-0.128	73	93
PIP2	0.146	0.405	9	3
TAG	0.189	-0.405	7	14
HexCer	0.201	-0.090	112	133
PE	0.247	-0.118	56	70
PS	0.247	0.091	92	76
MAG	0.294	-0.116	49	61
Sulfatide	0.371	0.145	26	19
Cer	0.453	0.357	5	2
CE	0.488	0.109	29	23
Cardiolipin	0.774	-0.182	5	7
GM3	0.839	-0.087	11	13
DAG	0.878	-0.049	20	22

Figure 4. Inhibition of FASN alters tumor metabolism in PDX models identified as sensitive to TVB-3664. Changes in tumor **(A)** and plasma **(B)** metabolites in Pt 2402, Pt 2449 PT and Pt 2614 identified as sensitive to FASN inhibition. **(C-D)** FASN-mediated changes in lipid classes common among Pt 2402, Pt 2449 PT and Pt 2614 PDX models. Lipid classes were evaluated by grouping the lipids to a class, and within each class setting lipids with fold-change > 0 as successes, and < 0 as failures, and testing the ratio of successes to failures to 0.5 using a two-sided binomial test. The reported value for the binomial test is the log-ratio of the calculated proportion of successes over 0.5.

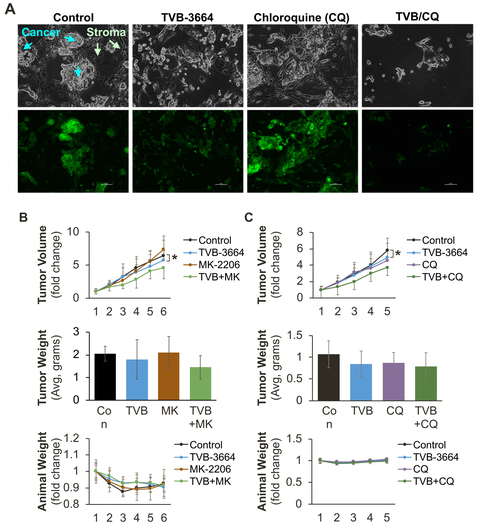


Figure 5. Anti-proliferative effect of combined inhibition of FASN and autophagy. Primary culture Pt #2387 treated with TVB-3664 (0.2 μM) and CQ (10 μM) for 7d. BF images and BODIPY493/503 stained cells for LDs presence are shown.

Summary

- FASN is overexpressed in CRC. 79% of CRC patients who had surgery at the University of Kentucky have high expression of FASN (immunoreactivity score >2);
- PDX models exhibit a wide range of sensitivity to FASN inhibition by TVB-3664 (3V-Biosciences);
- Inhibition of FASN by TVB-3664 in PDX models is associated with a significant decrease in abundance of FA, PA and PC in tumor tissues;
- A decreased pool of AXP is a common change among TVB-3664 treated tumors;
- The level of FASN expression may determine the sensitivity of tumors to FASN inhibition;
- Activation of Akt and autophagy pathways may be potential mechanisms of resistance to FASN inhibition.

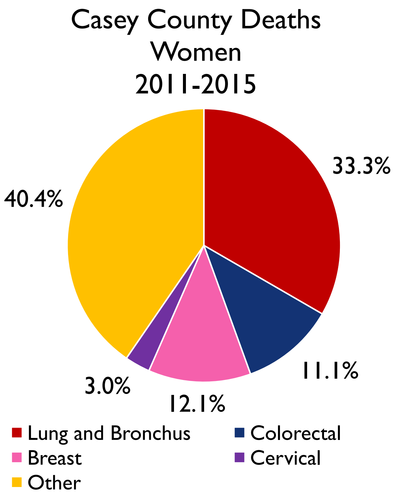
Conclusions

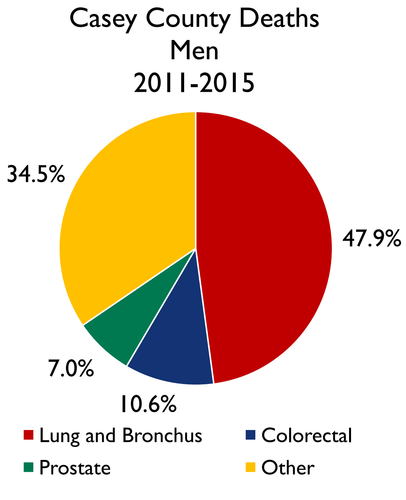
- Our studies show that the novel FASN inhibitors, as a single agent, significantly inhibit CRC growth both ***in vitro*** and *in vivo*.
- Targeted inhibition of FASN may represent a novel therapeutic strategy for treatment of CRC.
- The level of FASN expression and basal activation of Akt and AMPK may be predictive of responsiveness to FASN inhibition and may function as potential biomarkers to allow a more personalized treatment approach.
- Combination FASN and Akt inhibitors may be beneficial in selected tumors.

Acknowledgements

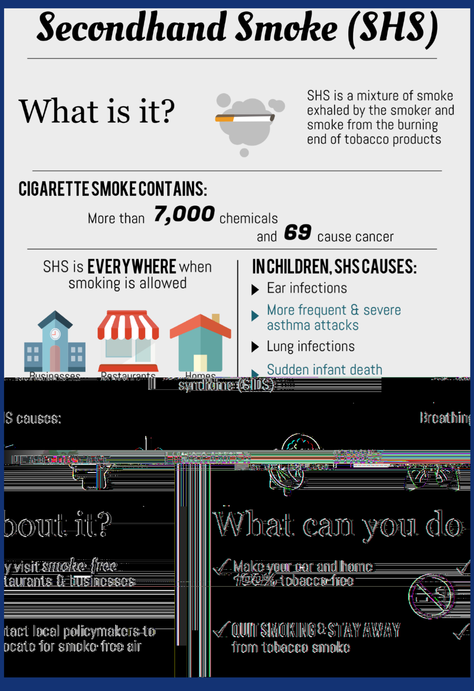
This work is supported by grants from 3-V BIOSCIENCES, INC. and the American Cancer Society IRG 85-001-25. The University of Kentucky Markey Cancer Center's Research Communications Office assisted in preparation of this poster.

Together, Our Workplace Can Help Reduce Lung Cancer in Casey County

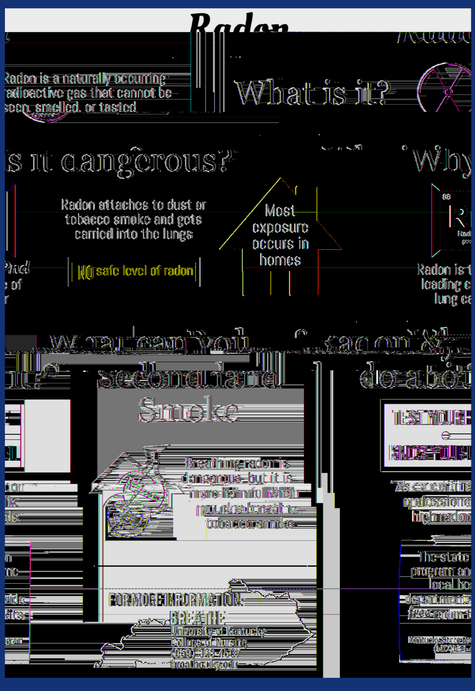




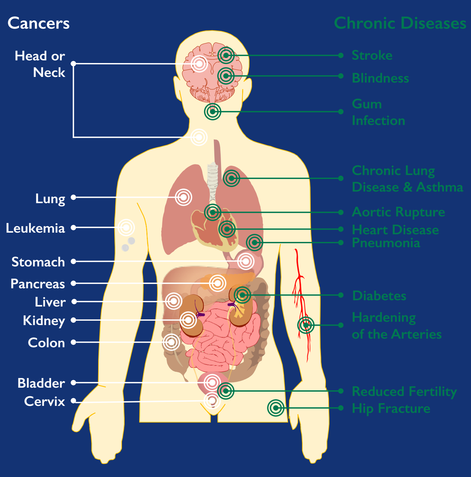
Reduce Exposure to Secondhand Smoke



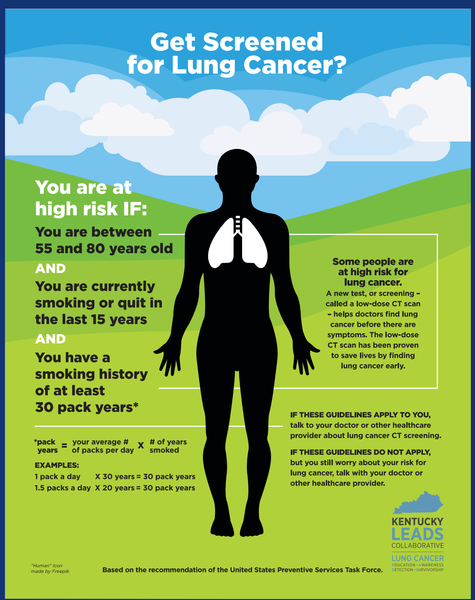
Reduce Exposure to Radon



Reduce Tobacco Use



Talk to Your Doctor About Cancer Screening



This poster was supported by Grant or Cooperative Agreement Number, NU58DP006313 (Kentucky Cancer Consortium) and DP13-1314 National Networks to Reduce Cancer and Tobacco Related Disparities (SelfMade Health Network) funded by the Centers for Disease Control and Prevention.

Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Cancer Data from the Kentucky Cancer Registry: www.kcr.uky.edu

College of Public Health

KCP Kentucky Cancer Program

Kentucky Cancer Consortium
A partnership for life.

Markey Cancer Center Research Communications Office: A Centralized Research Administration Infrastructure Enhancing Faculty Productivity

Heather N. Russell-Simmons, Cathy Anthony, Marcia Ballard, Jonathan Coffman, Donna Gilbreath, Terry L. Keys, Danielle Story, Jennifer F. Rogers, Jennifer Bybee, David M. Gosky, Nathan L. Vanderford

Markey Cancer Center, University of Kentucky, Lexington, Kentucky

HealthCare MARKEY CANCER CENTER
An NCI-Designated Cancer Center

University of Kentucky

Background

The University of Kentucky's (UK) Markey Cancer Center (MCC) is a premier cancer research center and patient care facility. Markey's basic, translational and clinical research supports its mission to reduce cancer incidence and mortality in Kentucky, where cancer rates are extraordinarily high. Cancer center programs include research, treatment, education and community engagement, focused on the underserved population of Appalachian Kentucky.

In support of this wide-ranging mission, the Research Communications Office (RCO) was created in 2009 to help cancer researchers obtain grant funding and publish material in support of their research. Since its creation, RCO's scope has grown to encompass pre-award and budgetary planning, event planning, and coordination with Markey's public relations and marketing personnel.

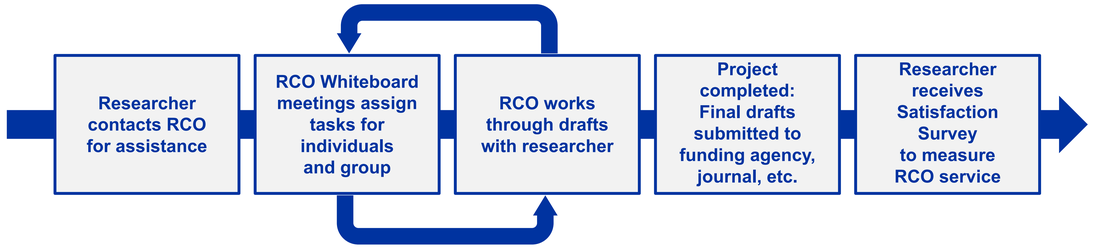
The framework and philosophy of this centralized research administration infrastructure serves as a case study on efforts to enhance faculty productivity.

RCO Staff and Responsibilities

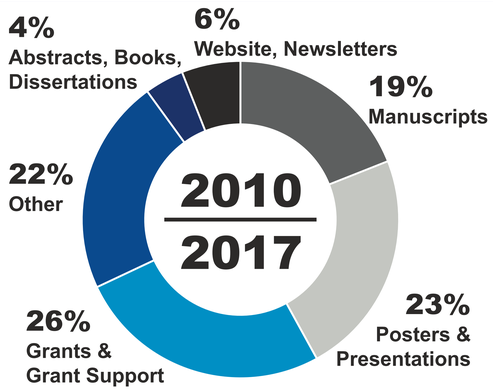
MCC RCO Staff and Responsibilities.

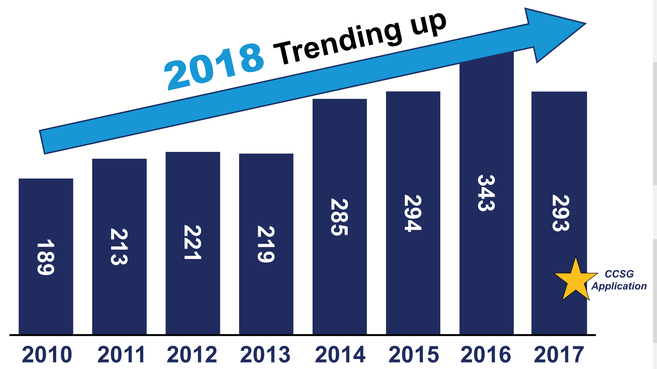
Position/Title	Responsibilities
Manager	• Daily management of personnel and budget • Serve as the key point of contact for the office, often helping initiate new projects and communicate RCO skills and expertise • Lead project coordinator and manager for proposal development, especially for multi-college and/or multi-department interactions • Manage the solicitation and peer-review process for all MCC developmental research projects/pilot funds • Lead tracking and routine reporting efforts for the cancer center (for example, membership and publication output)
Pre-Award Specialist	• Liaison between MCC and UK College of Medicine Sponsored Research Administrative Services and UK Office of Sponsored Projects Administration • Assist researchers in identifying funding opportunities • Ensure compliance with funding opportunity guidelines • Disseminate information about guideline changes for major grant sponsors • Budget development for large multi-component projects • Coordinate completion of data tables and institutional information components for training grant and career development applications
Editor/Designer	• Editing grants and manuscripts for grammar, content and compliance • Creation and editing of images and figures • Project management for small grants
Web Editor	• Maintenance and content creation for MCC website and social media • Design and distribution of newsletters • Web project liaison to UK HealthCare Marketing and Public Relations
Education Liaison	• Weekly Markey Research Seminars • Patient Advisory Group • Cancer conferences • Markey Cancer Center Research Day
Project Coordinator	• Coordination of developmental funds program • Evaluation of funding initiatives • Track, evaluate and report on pilot awards and funded projects

RCO Process



Projects





Campus Diversity of RCO Use

9 of UK's 16 Colleges used RCO services between 2010 – 2017.

39 UK Departments used RCO services between 2010 – 2017.

RCO Recognition

Markey Difference Maker of the Year 2012 and 2013, Finalist 2016

Society of Research Administrators International's 2016 Symposium "Best Poster of the Year Award North America"

Russell-Simmons HN, Anthony C, Ballard M, Coffman J, Gilbreath D, Keys TL, Story D, Rogers JF, Gosky DM, Vanderford NL. Enhancing faculty productivity through a centralized communications and project management infrastructure: a case study at the University Of Kentucky Markey Cancer Center. J Res Adm 47:68-79, 2017.

RCO Services

MCC RCO Project Type and Services Offered.

Project Type	Service Offered
Grants	Ensure all funding organization guidelines are met, review for correct grammar and spelling, check organization, confirm formatting consistency among the various components, including the budget, text and graphics. Complex grants require coordination among several entities with the RCO as a planning hub, providing timelines, organization, centralized communication, and acting as a clearinghouse for communication.
Manuscripts and Book Chapters	Review formatting for adherence to publisher guidelines, check for grammar, spelling and consistency, ensure that writing is clear and concise, verify reference style, review graphics for quality, improve or redraw figures as needed, provide figures in appropriate resolution and file format, submit text and graphic files to the selected journal or publisher, reviews proofs, and collect copyright forms as needed.
Presentations	Assistance with an oral presentation or poster by editing and submitting attendee abstract, reviewing preliminary slides or posters, improving figures, condensing text, adding animation where appropriate and ensuring consistent style throughout a slide presentation.
Internal Communications	Writing and distributing the Markey Minute, a weekly newsletter that provides a single, encompassing news source covering a weekly calendar of tumor boards, seminars, speakers, events and news. Writing and distributing the Markey Quarterly, a newsletter that provides a more in-depth exploration of the people and accomplishments at MCC. PDFs copies of the quarterly newsletter archived online.
Website	Maintain and create content for the MCC website and social media. Serve as web project liaison for UK HealthCare Marketing and Public Relations.
Pre-Award	Coordinate submission of information requested by sponsors prior to award, work with researchers to ensure that IRB, IACUC, and biosafety approvals are obtained prior to award, provide revised budget or other information to the UK Office of Sponsored Projects Administration for account establishment.
Event Planning	Coordinate research-related events such as Markey Cancer Research Day, weekly seminars, Grand Rounds and patient-centered education.

Lessons Learned

Secure critical buy-in from leadership. Long-term success of institutional research communication services is dependent on the support of leaders at the highest levels of the organization.

Use metrics and quality measures to reveal areas of unmet need. Metrics and quality measures provide important insight about services offered, as well as gaps in those offerings.

Emphasize a team-oriented culture. Hire staff who excel with individual challenges but also fully embrace highly collaborative environments.

Conclusions

We have presented an infrastructure that successfully enhances research productivity at our institution. Based on our experiences, we believe the ability to provide research communication services, and to grow and meet identified needs among researchers, is a unique opportunity for other institutions.

Cancer Beliefs and Perceptions among a Sample of Appalachian Kentucky Residents as Compared to National HINTS Respondents

Robin C. Vanderpool, DrPH, CHES; Quan Chen, DrPH; Meghan Johnson, MPH; Autumn Rice; Kristin O'Leary; Lauren Shelton, BS; Devin Montgomery

University of Kentucky Markey Cancer Center, Lexington, Ky.

HealthCare MARKEY CANCER CENTER
An NCI-Designated Cancer Center

NCI Cancer Center
A Cancer Center Designated by the National Cancer Institute

Background

Appalachian Kentucky communities experience increased socioeconomic disparities and an undue burden of cancer, particularly malignancies that are preventable and screenable such as lung, colorectal, breast, and cervical cancer. Understanding Appalachian residents' perceptions of cancer is important for designing health communication messaging and educational programming to improve cancer health literacy and decrease related disparities.

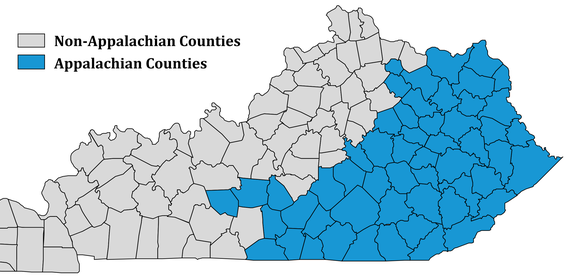
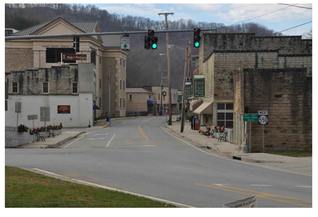


Figure 1. Kentucky map highlighting Appalachian counties. These are the 54 counties administered the health survey.



Methods

As part of an administrative supplement from the National Cancer Institute, the University of Kentucky Markey Cancer Center developed and administered a health-related survey – ASK: Assessing the Health Status of Kentucky – during summer 2017 in the 54 counties of eastern Kentucky designated as Appalachian. Survey items assessed a range of topics including: cancer information-seeking, cancer fatalism, cancer knowledge and beliefs, cancer screening and other health behaviors (e.g., tobacco use), and socio-demographics. Data collection methods included a probabilistic address mailing (N=3200) and community-based, in-person administration (N=200). Herein, we report on preliminary findings from the (unweighted) mailed sample (n=786, 25% response rate) as compared to national (unweighted) data from NCI's Health Information National Trends Survey (HINTS) 3 and 4 related to perceptions of and beliefs about cancer.

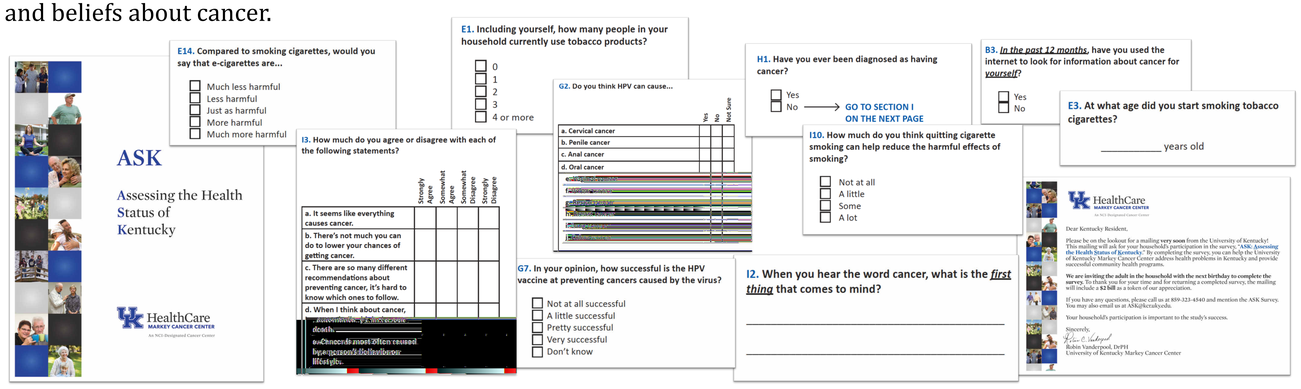


Figure 2. Assessing the Health Status of Kentucky (ASK) Survey. Excerpts from the survey relating to beliefs about cancer.

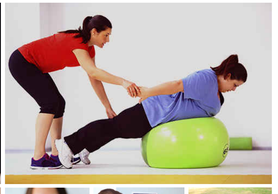
Results

Compared to national HINTS respondents, Appalachian Kentuckians were more likely to strongly or somewhat agree that:

- "Everything causes cancer" (59% vs. 67%, respectively)
- There's not much one can do to lower their chances of developing cancer (27% vs. 32%)
- Perceive that there are too many different recommendations about preventing cancer (74% vs. 80%)
- Automatically equate cancer to death (53% vs 71%)
- Would rather not know their chances of developing cancer (31% vs. 38%)

Conclusion

Based on these initial results, Appalachian residents commonly reported unfavorable perceptions of and beliefs about cancer that may influence their self-efficacy and locus of control related to behaviors that may help prevent or screen for cancer. Tailored, culturally-appropriate health communication messaging that (1) promotes the power of preventive behaviors (e.g., physical activity, healthy diet, tobacco cessation) and screening exams (e.g., mammography, colonoscopy, low-dose CT scans, Pap testing) to improve cancer outcomes and (2) provides factual information about increasing survival rates for all cancer sites may help to address Appalachian's negative beliefs about cancer.



Acknowledgement

This research was supported by the Behavioral and Community-Based Research, Biostatistics and Bioinformatics, and Cancer Research Informatics Shared Resource Facilities of the University of Kentucky Markey Cancer Center (P30 CA177558) and funded by NCI Administrative Supplement: P30 CA177558-04S5. The UK MCC Research Communication Office assisted with poster development.