BIOGRAPHICAL SKETCH

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NAME: Langenfeld, John, M.D.

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

POSITION TITLE: Associate Professor

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)	END DATE MM/YYYY	FIELD OF STUDY
South Dakota State University	BS	07/77-06/82	Microbiology
University of South Dakota		07/83-06-85	Medicine
Rush Medical School, Chicago, IL	MD	08/85-06/87	Medicine
UMDNJ-New Jersey Medical School	Resident	07/87-06/93	Surgery
UMDNJ-New Jersey Medical School	Research	07/88-06/89	Sepsis
Memorial Sloan-Kettering Cancer Center	Research	07/94-06/97	Chemoprevention
West Virginia University	Resident	07/97-06/99	Thoracic Surgery

A. Personal Statement

I am a physician scientists working at University of Medicine and Dentistry of New Jersey (Now Rutgers University) as a thoracic surgeon and independent researcher since 1999. I have been studying the role of bone morphogenetic proteins (BMP) in cancer for the last 21 years. I performed a cDNA subtraction assay and identified that bone morphogenic protein 2 (BMP-2) is expressed in non-small cell lung carcinomas (NSCLC). BMP-2 was identified to be highly over-expressed in 98% lung carcinomas with little expression in normal lung tissue. My laboratory is the first to report that aberrant BMP expression in cancer cells is tumorigenic. We discovered that BMP-2 ligand stimulates tumor migration, metastasis, and angiogenesis. Subsequently, multiple labs have corroborated our results in many different types of cancers. We have found that the BMP type 1 (BMPR1) and type 2 receptors (BMPR2) have uniquely different functions regulating cell survival. I established a group of scientists that includes medicinal chemistry, synthetic chemistry, computational biology, crystallography, BMP biologist, and pathology, who all work at Rutgers, to develop BMPR2 inhibitors. We have been working as a group for over 5 years. We published that the BMP inhibitor JL5, which we designed, promotes tumor regression in mice without toxicity. This work further highlights the importance of BMP signaling in cancer and has established the medicinal chemistry platform to design more specific BMPR2 inhibitors for cancer and other BMP related diseases.

Bone morphogenetic protein (BMP) signaling is a phylogenetic conserved regulator of embryonic development. BMP signaling becomes silent in most organs post development. However, BMP signaling is reactivated in several age-related diseases including cancer, Alzheimer's disease, obesity, and cardiovascular disease. Our lab has revealed that conserved signaling events mediated by the BMP type 2 receptor (BMPR2), which are required for development and are also present in lung cancer. This includes the regulation of X-linked inhibitor of apoptosis (XIAP) and the stabilization of the microtubules. We recently published, showing for the first time, that BMP signaling suppresses catabolic metabolism and stimulates anabolic metabolism in lung cancer cells, which is conserved in *C elegans*. The combination of BMPR2 inhibitors with mitochondria targeting agents (Ym155 and phenformin), induces synergistic cell death and nuclear localization of apoptosis inducing factor (AIF) independent of caspases. AIF is an evolutionary conserved cell death pathway that occurs independent of apoptosis. The combination of Navitoclax and BMPR2 inhibitors synergistically increase $mtCa^{++}$ levels, AIF nuclear localization, and cell death of lung cancer cells. These studies suggest that BMPR2 inhibition can uniquely promote cell death that utilizes evolutionary conserved cell death pathways. Consistent with this hypothesis, our recent preliminary studies show that BMPR2 regulates VDAC1. VDAC1 a phylogenetic conserved master regulator of both cell survival and cell death and the gatekeeper of mitochondrial calcium levels. Our group has made significant progress developing potent and specific BMPR2 inhibitors that can be made into a drug. At this point in my career, the majority of my time is devoted to this research project. Our entire team is committed to bringing BMPR2 targeted therapy into the clinic as a novel treatment strategy for cancer.

B. Positions and Honors

Positions:

1993	Administrative Chief Resident, General Surgery, UMDNJ-New Jersey Medical School
1999-present	Rutgers, Robert Wood Johnson Medical School and Rutgers Cancer Institute of New Jersey, Department of Surgery
2002-2020	Section Leader Thoracic Surgery, Rutgers Robert Wood Johnson Medical School
2012-2020	Co-Director of Thoracic Oncology, Rutgers CINJ.
2020-present	Chief, Thoracic Surgery

Selected Honors:

1987	Cardiology Prize, Outstanding student performance. Rush Medical College, Chicago IL
1988	Outstanding Resident's Research Project, American College of Surgeons, NJ Chapter
2006-2021	Top Doc, New Jersey Monthly
2006-2013	Rank #1 in NJ and 24th in the country for Respiratory Diseases by U.S News and Report among the top ranked surgeons for outcomes.

Selected Additional Professional Memberships/Service:

Fellow, American College of Surgeons
Society of University Surgeons
American Association for Cancer Research
American Thoracic Society
Association for Academic Surgery
General Thoracic Surgical Club

C. Contribution to Science

1. Examining chemoprevention mechanisms: My initial oncology research in Ethan Dmivstrosky's laboratory was the study of the mechanisms by which all-trans-retinoic acid regulated bronchial epithelial cells as it related to its chemopreventive properties for lung cancer. The challenges at this time (1994) were to identify the optimal retinoid for chemoprevention, determine their mechanism of action, and provide clinically useful biomarkers. I developed an *in vitro* model of transformation using tobacco-derived carcinogens, cigarette smoke condensate (CSC) and N-nitroamine-4-(methylnitrosamino)-1-(3 pyridyl)-1-butanone (NNK). All-trans-retinoid acid (RA) inhibited this transformation, which was associated with a downregulation of cyclin E. We also showed that forced expression of cyclin E enhanced proliferation of BEAS-2B cells suggesting its role in early transformation. I conceived and carried out these experiments. This was the first study showing a chemopreventive agent regulated cell cycle proteins. My subsequent paper showed that the mechanism RA regulated the expression of D1 occurred through ubiquitin proteasome dependent proteolysis. This work was published in PNAS, which I was the corresponding author.

Other studies relating to my work that I contributed to include the demonstration that over-expression of G1 cyclins occurred early in transformation of lung cancer. We also demonstrated that receptor-nonselective retinoids were superior to carotenoids in regulating the proteolysis of cyclin D1. Clinical trials have demonstrated the importance of downregulating the expression cyclin D1 to evaluate the effectiveness of chemopreventive agents.

a. **Langenfeld, J.**, Lonardo, F., Rusch, V., Dmitrovsky, E. Retinoic acid down-regulates cyclin E and inhibits *in vitro transformation* of immortalized human bronchial epithelial cells. Oncogene, 1996; 13; 1983-1990.
b. **Langenfeld, J.**, Kiyokawa, HK., Sekula, D., Boyle, J., and Dmitrovsky, E. Posttranslational regulation of cyclin D1 by retinoic acid: a chemoprevention mechanism. Proc Natl Acad Sci USA, 1997; 94; 12070-74.
c. Lonardo, F., **Langenfeld, J**., Rusch, V., Dmitrovsky, E., and Klimstra, D. Aberrant expression of cyclin D1 and E, but not Rb is frequent in bronchial preneoplasia and precedes squamous cell carcinoma development. Cancer Research 1998; 59 (10); 2470-76.
d. Boyle, J., **Langenfeld, J.**, Lonardo, F., Reczek, P., Rusch, V., Dawson, M., and Dmitrovsky, E. Cyclin D1 proteolysis is a retinoid cancer chemopreventive signal in normal, immortalized, and transformed human bronchial epithelial cells. J of National Cancer Institute, 1999; 91(4); 373-79.

2. **Identifying that BMP2 is uniquely expressed in lung cancer and examining its role in cancer:** I had an interest in pursuing genes uniquely expressed in primary lung carcinomas that may regulate metastasis. After arriving at UMDNJ (now Rutgers), I used representational difference analysis to evaluate uniquely expressed genes in a primary lung adenocarcinoma compared to immortalized bronchial epithelial cells (BEAS-2B cells). This analysis revealed that bone morphogenetic protein 2 was expressed in primary lung carcinomas but not

primary lung tissue, BEAS-2B cells, or benign lung tumors (Langenfeld 2003). BMP-2 had only been detected in cancer cell lines by PCR but its expression in primary tumors had never been demonstrated. At this time, there was no data demonstrating that BMP signaling had a biological role in cancer. We showed that BMP-2 enhanced migration and invasion of lung cancer cells (Langenfeld 2003). Co-injection of recombinant BMP-2 with A549 cells enhanced tumor growth while the BMP-2 antagonist noggin decreased tumor growth (Langenfeld 2003). This work provided the first reported evidence demonstrating that BMP-2 is aberrantly expressed in primary tumors and may promote tumorigenesis.

Examining a larger cohort of patient samples, we found that BMP-2 is highly over-expressed in 98% of all lung cancer including adenocarcinomas, squamous, and neuroendocrine tumors (Langenfeld 2005). We found that BMP-2 enhanced neovascularization of developing tumors, which was inhibited with noggin and antisense BMP-2 cDNA (Langenfeld 2004). BMP-2 ligand also directly activated vascular endothelial cells. This was the first study demonstrating that BMP-2 enhanced tumor growth by stimulating angiogenesis. Since our initial publication in 2003 there have been numerous publications corroborating our results and supporting that the BMP signaling is growth promoting in many types of cancers.

a. Langenfeld, E.M., Calvano, S.E., Abou-Nukta, F., Lowry, S., Amenta, P., and **Langenfeld, J.** The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. Carcinogenesis, 2003; 24 (9) 1445-54. PMID: 12819188
b. Langenfeld EM, **Langenfeld J**. Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. Mol Cancer Res, 2004;2(3):141-9. PMID: 15037653
c. Elaine M. Langenfeld, John Bojnowski, John Perone, and **John Langenfeld**, Expression of Bone Morphogenetic Proteins in Human Lung Carcinomas, Ann. Thorac. Surg, 2005; 80: 1028 – 1032. PMID: 16122479

3. Discovering that BMP signaling promotes tumorigenesis: At the time little was known how BMP regulated cancer cells and whether it was promoting or suppressing tumorigenesis. There were conflicting studies suggesting that BMP signaling may be a tumor suppressor. Based on what we knew of BMP signaling at the time, we hypothesized that the methods by which BMP signaling was studied would significantly affect the results. We found that BMP signaling regulated the expression of the oncogene, inhibitor of differentiation protein 1 (Id1) in lung cancer cells. Forced expression of BMP-2 in A549 cells greatly enhanced metastatic tumor growth in the lungs associated with increased expression of Id1, while in subcutaneous tumor it suppressed tumor growth and decreased Id1 expression (Langenfeld 2006). These studies support that BMP signaling is growth promoting and was dependent on the microenvironment and highlighted the importance of its activation of Id1. Our work also found that BMP-2 regulates the PI 3-kinase/mTOR signaling pathway in lung cancer (Langenfeld 2005). Utilizing siRNA and small molecule inhibitors, we learned that lung cancer cells signal through all type I receptors (alk2, alk3, and alk6) to regulate Id1 and Id3. Our data showed that inhibition of all of the type I receptors was required to suppress growth and induce death of lung cancer cells, which was mediated in part by the downregulation of Id proteins (Langenfeld 2013). We developed an *in vitro* model to analyze cancer stem cells by isolating from cell lines, cells that activated the Oct4 or nestin promoter. We found this to be an interesting model because we could isolate highly tumorigenic and unique populations of cancer cells that also demonstrated differentiation. This published work showed that suppressing BMP signaling inhibited the growth of lung cancer stems cells (Langenfeld 2013). These studies provided the first definitive evidence demonstrating that BMP signaling is growth promoting and enhanced the survival of cancer cells.

a. Langenfeld, Elaine; Kong, Yingxin; **Langenfeld, John**; Bone Morphogenetic Protein-2 Stimulation of Tumor Growth Involves the Activation of Smad 1/5. Oncogene, 2006; 25(5):685-92. PMID: 16247476
b. Elaine M. Langenfeld, Yingxin Kong, and **John Langenfeld**, Bone Morphogenetic Protein-2 Induced Transformation Involves the Activation of Mammalian Target of Rapamycin Mol. Cancer Res, 2005; 3 (12) 679-684. PMID: 16380505
c. Elaine Langenfeld, Gandhi Lanki, Charles Hong, **John Langenfeld**. Bone Morphogenetic Protein Type I Receptor Antagonists Decrease Growth and Induce Cell Death of Lung Cancer Cell Lines. PloS One, 2013; 8(4): e61256. PMID: 23593444
d. Elaine Langenfeld, Malik Deen, Emmanuel Zachariah, **John Langenfeld.** Small molecule antagonist of the bone morphogenetic protein type I receptors suppresses growth and expression of Id1 and Id3 in lung cancer cells expressing Oct4 or nestin. Molecular Cancer, 2013, 12:129. PMID: 24160469

4. Developing BMP receptor inhibitors and BMP type 2 receptors have an unique role regulating growth of cancer cells. We continued to examine the role of the BMP type 1 receptor promoting tumorigenesis. We

found a feedback mechanism by which suppression of TGFβ increased Id1 expression through the activation of TAK1 (Augeri 2016). On further exploration we discovered that BMP type 2 receptors upregulated the expression of the anti-apoptotic protein XIAP, which controls the regulation of TAK1 (Augeri 2016). This was our first insight that BMPR2 may have an important role regulating survival of cancer cells. In collaboration with medicinal chemists at Rutgers, we began developing small molecule inhibitors of BMP receptors. All BMP inhibitors at the time targeted BMP type 1 receptors. We identified that one of the BMP inhibitors (DMH2) being studied was quickly oxidized becoming unstable. We modified DMH2 so it was more stable creating JL5 (Newman 2018). JL5 was more stable so we were able to perform mice xenograft studies. JL5 suppressed growth of tumor xenografts in mice (Newman 2018). This suggested that BMP signaling could be targeted in cancer with small molecules. Further exploration revealed that JL5 had an unique property compared to other BMP inhibitors in that it induced trafficking of BMPR2 from the plasma to the cytosol thereby decreasing activity (NeMoyer 2019). BMP inhibitors that suppressed BMPR2 were significantly more potent in decreasing BMP signaling and suppressing survival of cancer cells compared to inhibitors that only targeted type 1 receptors (Augeri 2016). Subsequently, we found that the inhibition of BMPR2 increased mitochondrial release of Smac/DIABLO decreasing XIAP expression that sensitized lung cancer cells to TRAIL (NeMoyer 2019). These studies suggested that BMPR2 promotes survival that is independent of BMP type 1 receptors.

a. Dave Augeri, Elaine Langenfeld, Monica Castle, John Gilleran, **John Langenfeld**. Inhibition of BMP and TGFβ receptors downregulates expression of XIAP and TAK1 leading to lung cancer cell death. Molecular Cancer. 2016 Apr 6;15:27. PMID: 27048361
b. Jenna H. Newman, David J. Augeri, Rachel NeMoyer, Jyoti Malhotra, Elaine Langenfeld, Charles B. Chesson, Natalie S. Dobias, Michael J. Lee, Saeed Tarabichi, Sachin R. Jhawar, Evita T. Sadimin, John E. Kerrigan, Michael Goedken, Christine Minerowicz, Salma K. Jabbour, Shengguo Li, Andrew Zloza & **John Langenfeld.** Novel bone morphogenetic protein receptor inhibitor JL5 suppresses tumor cell survival signaling and induces regression of human lung cancer. Oncogene, 2018 July;37(27):3672-3685. PMID: 29622797
c. Targeting bone morphogenetic protein receptor 2 sensitizes lung cancer cells to TRAIL by increasing cytosolic Smac/DIABLO and the downregulation of X-linked inhibitor of apoptosis protein. Rachel NeMoyer, Arindam Mondal, Mehul Vora, Elaine Langenfeld, Danea Glover, Michael Scott, Lauren Lairson, Christoper Rongo, David J. Augeri, Youyi Peng, Salma K. Jabbour, **John Langenfeld.** 2019 Cell communication and Signaling. 2019 Nov 19;17(1)150. PMID: 31744505

5. Developing BMPR2 inhibitors and BMPR2 regulation of energy homeostasis. Over the last 4 years, we have developed potent and specific BMPR2 inhibitors (in vitro IC50 for BMPR2 of 20 nM). Some of the BMPR2 inhibitors demonstrate no inhibition of the type 1 receptors. The work from the R01 has led to a provisionary patent application to cover our novel BMPR2 inhibitors (March-21-22-#63/156,423). Our studies show for the first time that BMPR2 is integrated with both catabolic and anabolic metabolism. BMPR2 promotes anabolic signaling through the activation of PI3K (Vora 2022). BMPR2 suppresses catabolic signaling by inhibiting AMP activated kinase (AMPK) through its inhibition of LKB1 (Vora 2022). We found that that AMPK causes a negative feedback regulation of BMPR2 while PI3K causes a positive feedback regulation of BMPR2 through Akt (Vora 2022). These data suggest that aberrant BMP signaling through BMPR2 causes dysregulation of metabolism. Dysregulation of metabolism produces a pathological responses in several age-related disease including cancer, AD, and obesity.

Inducing synergy to promote death of cancer cells. We have an ongoing research effort to understand how best to induce synergy with BMPR2 inhibitors to promote death of cancer cells. We demonstrated synergistic cell death with several BMPR2 inhibitors when combined with Ym155. The mechanism of this synergy was not clear because the mechanism of Ym155 was poorly understood. We found that Ym155 binds mitochondria DNA, which significantly decreases mitochondrial function (Mondal 2022). Synergistic cell death was found to involve the “hyperactivation” of AMPK. The hyperactivation of AMPK was found to decrease BMP signaling by inhibiting of BMPR2 (Mondal 2022). The hyperactivation of AMPK was also found to increase the nuclear localization of apoptosis inducing factor (AIF) (Mondal 2022). AIF mediates a cell death pathway that occurs independent of apoptosis, which is frequently cause by high mitochondrial Ca^{++} levels ($mtCa^{++}$). The mitochondrial complex 1 inhibitor phenformin, also enhanced AIF nuclear localization and cell death when combined with BMPR2 inhibitors (Mondal 2022). An interesting observation was that synergy involves an increase in $mtCa^{++}$ levels. Because BCL-2 and BCL-xL inhibits apoptosis and influx of $mtCa^{++}$, we examined synergy of BCL-2/BCL-xL inhibitors with BMPR2 inhibitors. Preliminary studies show significant synergistic cell death using the BCL-2/BCL-xL inhibitor Navitoclax together with our BMPR2 inhibitors. Cell death is associated

with an increase in $mtCa^{++}$ levels, AIF nuclear localization, and apoptosis.

Our studies also suggest that BMPR2 regulation of microtubules is involved in the synergistic cell death process. BMPR2 is known to stabilize the microtubules. We found that BMPR2 inhibition, with siRNA and with multiple BMPR2 inhibitors, destabilizes the microtubules (Mondal 2021). The combination of Ym155 and BMPR2 inhibitor JL5 increased lysosome permeabilization during cell death (Mondal 2021). Our preliminary studies now suggest that the destabilization of the microtubules promotes the localization of voltage dependent anion channel (VDAC) to the mitochondria. VDAC is the gatekeeper of $mtCa^{++}$ levels and is a phylogenetic conserved master regulator of cell survival and cell death. This finding has implications into the mechanisms by which BMPR2 inhibition can enhance mitochondrial function but when combined with Navitoclax promotes cell death of cancer cells. This line of research will be pursued in the R01 renewal.

a. Inhibition of BMPR2 destabilizes the microtubules initiating cell death of cancer cells by activating lysosomes. Arindam Mondal, Rachel NeMoyer, Elaine Langenfeld, John Gilleran, Youyi Peng, Jacques Roberge, Mehul Vora., **John Langenfeld.** Cell Communication and Signaling. 2021. Sep 25;19(1)97. PMID: 34563224
b. Bone Morphogenetic protein signaling regulation of AMPK and PI3K in lung cancer cells and *C. elegans* Mehul Vora, Arindam Mondal[1], Dongxuan Jia, Pranya Gaddipati, Moumen Akel, John Gilleran, Jacques Roberge, Christopher Rongo, **John Langenfeld**. Cell Biosci. 2022. May 31;12(1):76. PMID: 35641992
d. Ym155 localizes to the mitochondria leading to mitochondria dysfunction and activation of AMPK that inhibits BMP signaling in lung cancer cells. Arindam Mondal, Dongxuan Jia, Vrushank Bhatt, Moumen Akel, Jessie Yanxiang Guo, **John Langenfeld**. Sci Rep. 2022 Jul 30;12(1):13135. PMID: 35908087
e. Bone morphogenetic protein inhibitors and mitochondria targeting agents synergistically induce Apoptosis-Inducing Factor (AIF) caspase-independent cell death in lung cancer cells. Arindam Mondal, Jacques Roberge, John Gilleran, Youyi Peng, Dongxuan Jia, Moumen Akel, Yash Patel Harrison Zoltowski, Anupama Doraiswamy, **John Langenfeld**. Cell Commun and Signal. 2022 Jun 27;20(1):99. PMID: 35761398

D. Additional Information: Research Support and/or Scholastic Performance

Current Research Support:

RO1 CA225830-01A1-Langenfeld (PI) July 1, 2018 – June 29, 2023
National Institute of Health, NCI $ 230,000 annual direct cost
Developing bone morphogenetic receptor II inhibitors for the treatment of cancer
Develop novel BMPRII inhibitors and characterize unique mechanisms by which these inhibitors induce cell death in comparison to inhibition of the type I BMP inhibitors.

HealthAdvance grant: Office of Rutgers commercialization Nov 1, 2020-Oct 2022.
and NIH $ 200,000 annual direct cost.
Langenfeld (PI)
Developing BMPR2 inhibitors for the treatment of cancer and Alzheimer's disease for Treatment of cancer and Alzheimer's disease.

3R01 CA225830-05S1-Langenfeld (PI) July 1, 2022-June 29, 2023
National Institute of Health $ 249,144 direct cost
Determining the mechanism by which bone morphogenetic protein inhibition promotes survival/neurogenesis and to trigger AMPK hyperactivation that includes neuronal and cancer cell death.

R01CA225580- La Croix (PI), July 1, 2022-June 29, 2023
Mentor, Langenfeld $ 131,948 direct cost
National Institute of Health
Targeting BMP type 2 receptors for the treatment of breast cancer