THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Anja Bastian

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE
DEPARTMENT OF PHYSIOLOGY

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Mechanism of Action of a Novel Small Molecule, AG311, for Triple Negative Breast Cancer Therapy

COMMITTEE IN CHARGE: Michael A. Ihnat, Ph.D., Chair; Robert D. Foreman Ph.D.; Kennon M. Garrett, Ph.D.; William E. Sonntag, Ph.D. Zhongjie Sun, Ph.D.; Wei-Qun Ding, Ph.D.

ABSTRACT:
Treatment of triple negative breast cancer (TNBC) is difficult due to its aggressive and metastatic nature, leading to high recurrence and mortality rates. The targeted and endocrine treatments used for other types of breast cancers are largely ineffective for TNBC due to the absence of the target receptors (estrogen, progesterone, Her2/neu receptors). TNBC is also prone to develop resistance to traditional chemotherapies and patients are left with large ineffective therapeutic options. Therefore, TNBC represents an important clinical challenge to develop more effective agents with novel mechanisms of action and low systemic toxicity. The focus of this work was to identify and study the mechanism of a novel anticancer agent for the treatment of TNBC. A series of compounds was designed, synthesized, and evaluated for their dual-inhibition of growth factor receptors (EGFR, VEGFR-2, PDGFR-β) and inhibition of DNA synthesis (thymidylate synthase) in one single molecule. The compound AG311 (5-[(4-methylphenyl)thio]-9H-pyrimido[4,5-b]indole-2,4-diamine) was identified and tested in two mouse orthotopic TNBC models. AG311 reduced tumor volume (by 85%-81%), with no apparent systemic toxicity. Interestingly, we identified that AG311 induced rapid necrotic cell death, which could not be explained by its activities for

which it was designed. AG311 treatment was two-fold more selective for breast cancer cells versus noncancerous cells (IC_{50}-values: 6-14 µM vs 22-30 µM, respectively). Among the earliest AG311-induced cellular events were rapid ATP depletion and mitochondrial membrane depolarization, pointing towards the involvement of the mitochondria. Using *in vitro* kinetic studies of mitochondrial electron transport chain (ETC) activities, we identified complex I as a direct molecular target of AG311. Tumor tissue isolated from AG311-treated mice showed reduced oxygen consumption rate (OCR) as compared to solvent-treated mice in the presence of complex I substrates. Further, AG311 reduced HIF-1α stabilization under hypoxic conditions, likely as an effect of the reduced OCR. The identification of the mitochondria as a target for AG311 was rather unexpected, but has emerged as a therapeutic strategy with a recent increased understanding of cancer mitochondrial metabolism. This novel mechanism together with its *in vivo* efficacy data suggest that AG311 has a significant potential to become a novel therapeutic agent to treat TNBC.