Research proposal:

Title: Targeting NF-κB/Cyclin D1 axis to prevent endometrial cancer resistance to CDK4/6 inhibitors.

Currently, advanced endometrial cancer is being treated with carboplatin plus paclitaxel as a front-line treatment standard of care [1, 2]. However, increasing incidences of endometrial cancer demands for an alternative therapeutic option. Therefore, active clinical trials are investigating the potential of cyclin dependent kinases 4 and 6 (CDK4/6) inhibitor (abemaciclib) alone (NCT number: NCT04188548) and in combination with non-steroidal aromatase inhibitor (letrozole) (NCT number: NCT03675893) in advanced and recurrent endometrial cancers [3]. Recurrence of endometrial cancer are positively associated with chemoresistance, a high mortality rate [4]. Surprisingly, metastatic specimens in breast cancer patients were identified during treatment with CDK inhibitors (CDKIs), suggesting that several potential mechanisms are involved in primary or acquired resistance to CDKIs. Interestingly, in these resistant cells, Retinoblastoma 1 and AKT serine/threonine kinase 1 gene mutations or amplifications were most frequent observed [5]. Thus, studies are required to develop therapeutic strategies to improve chemotherapy response, and an alternative therapeutic strategy is needed to overcome chemoresistance against CDKIs, without increasing toxicity.

Abemaciclib treatment derived the nuclear factor kappa-B (NF-κB)-mediated upregulation of its downstream target in glioblastoma cells [6]. Activation of NF-κB transcriptional factor acquires chemoresistance by upregulating anti-apoptotic genes and ABC transporter expression [7, 8]. ABC transporters are well known to contribute drug resistance by increasing the efflux of drugs [9]. NF-κB was also upregulate *CCND1* transcription level [10]. *CCND1* is a gene which codes for the cyclin D1 protein. According to The Cancer Genome Atlas (TCGA) database, *CCND1* expression is significantly upregulated in a variety of cancers, including endometrial cancer. Interestingly, cyclin D1 amplification has shown a positive correlation with ABC transporter expression in several cancers [11]. Additionally, cyclin D1 is also known to participate in driving elevated cell cycle progression through its interaction with the CDK4/6 complex. Cyclin D1/CDK4/6 complex functionally deactivates the retinoblastoma family proteins and E2 transcription factors, thereby promoting cellular proliferation and malignant transformation [12]. Mortalin and hsc70 assure proper folding and function of cyclinD1/CDK4/6 complexes and components, thereby fostering the accelerated cellular proliferation driven by mutation and overexpression of these oncoproteins in cancer cells [13-15]. In addition, researchers evidenced that abemaciclib essentially blocks the CDK4/CDK6-Cyclin D1 complex formation, but it does not essentially deregulate cyclin D1 protein expression [16]. In fact, abemaciclib resistant cancer cells have shown an increased Cyclin D1 expression [17]. Researchers in the field have found that free cyclin D1 can modulate histone deacetylases (HDACs) activity independent of CDKs [18, 19]. Histone deacetylases is very well known to simulate response against immune therapy [20]. Endometrial cancer is characterized by genetic and epigenetic mutations that increase susceptibility to CDK4/6 inhibitors, especially in cancers with mutations in the cyclin D1 gene 3' UTR [13, 14]. Mutations in *CCND1* are predicted to activate CDK4/6 complexes by inhibiting cyclin D1 phosphorylation at Thr286, block its nuclear transport and prevent its degradation, which further facilitates tumor growth and progression [21]. Endometrial cancer is one of the most sensitive cancers to CDK4/6 inhibitors in association with *CCND1* mutations [22]. Inhibition of cyclin D1 expression has shown to increase cisplatin chemosensitivity in human oral squamous cell carcinoma and corelates with NF-κB activity [23]. Similarly, another study in human pancreatic cancer cells demonstrated that overexpression of NF-κB-mediated cyclin D1 expression causes cisplatin resistance [7]. These studies strongly suggested the crucial role of NF-κB/*CCND1* axis in chemoresistance.

Thus, I hypothesize that resistant against CDK4/6 inhibitor is possibly upregulating NF-κB-mediated cyclin D1 expression to contribute endometrial cancer chemoresistance and may modulate the response against immune therapy. Therefore, NF-κB/cyclin D1 axis is implicated in chemoresistance justifying studies to validate its role in chemoresistance to identify vulnerabilities that can be targeted in development of strategies to prevent or overcome CDKIs chemoresistance in endometrial cancer.

I observed, treatment with abemaciclib, alone increases, while a novel investigational drug, SHetA2, decreases, NF-κB mediated cyclin D1 expression in endometrial Ishikawa cancer cells. I also found that these two drugs work synergistically in reducing endometrial cancer cell viability. SHetA2 treatment with palbociclib (a drug similar to abemaciclib) also effectively downregulated cyclin D1 level and CDK4/6 activity leading to reduced Rb phosphorylation [24]. Hence, studies of the mechanism and translational potential of abemaciclib and SHetA2 combination with a focus on how NF-κB/*CCND1* axis predict tumor response are warranted. Although, till today, there is no such evidence correlating NF-κB-mediated cyclin D1 expression modulating CDKIs drug sensitivity in endometrial cancer cells. Furthermore, the mechanistic role of SHetA2 combating CDKIs drug resistance has not been investigated extensively in endometrial cancers.

Overall, I hypothesize that pharmaceutical manipulation of NF-κB/*CCND1* axis can increase sensitivity of endometrial cancer cells to cyclin dependent kinase inhibitors.

Aim 1.1: Pharmaceutically manipulate NF-κB/*CCND1* axis to test its role in CDK4/6 inhibitor sensitivity and resistance *in vitro*. I will identify various doses and schedules of CDK4/6 inhibitors and SHetA2 for maximal killing of endometrial cancer cells with minimal effects on normal endometrial epithelial cells.

Aim 1.2: The optimal dose will then be evaluated for efficacy and toxicity *in vivo* against endometrial cancer xenograft model.

Aim: 1.3: Evaluating the synergistic efficacy of Cyclin D1 inhibitor in combination with HDAC inhibitor to suppress the progression of endometrial tumorigenesis.

The anticipated outcomes of this study are the identification of optimal dose and schedule of abemaciclib and chemotherapy for future clinical trials.

References:

1. Miller, D.S., et al., *Carboplatin and Paclitaxel for Advanced Endometrial Cancer: Final Overall Survival and Adverse Event Analysis of a Phase III Trial (NRG Oncology/GOG0209).* J Clin Oncol, 2020. **38**(33): p. 3841-3850.
2. Halla, K., *Emerging Treatment Options for Advanced or Recurrent Endometrial Cancer.* J Adv Pract Oncol, 2022. **13**(1): p. 45-59.
3. Konstantinopoulos, P.A., et al., *A Phase II, Two-Stage Study of Letrozole and Abemaciclib in Estrogen Receptor-Positive Recurrent Endometrial Cancer.* J Clin Oncol, 2022: p. JCO2200628.
4. Hellweg, R., et al., *RNA Sequencing of Carboplatin- and Paclitaxel-Resistant Endometrial Cancer Cells Reveals New Stratification Markers and Molecular Targets for Cancer Treatment.* Horm Cancer, 2018. **9**(5): p. 326-337.
5. Wander, S.A., et al., *The Genomic Landscape of Intrinsic and Acquired Resistance to Cyclin-Dependent Kinase 4/6 Inhibitors in Patients with Hormone Receptor-Positive Metastatic Breast Cancer.* Cancer Discov, 2020. **10**(8): p. 1174-1193.
6. Olmez, I., et al., *Combined c-Met/Trk Inhibition Overcomes Resistance to CDK4/6 Inhibitors in Glioblastoma.* Cancer Res, 2018. **78**(15): p. 4360-4369.

7. Biliran, H., Jr., et al., *Overexpression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line.* Clin Cancer Res, 2005. **11**(16): p. 6075-86.
8. Abdin, S.M., et al., *Nuclear factor-kappaB signaling inhibitors revert multidrug-resistance in breast cancer cells.* Chem Biol Interact, 2021. **340**: p. 109450.
9. Sun, Y.L., et al., *Role of ABC transporters in cancer chemotherapy.* Chin J Cancer, 2012. **31**(2): p. 51-7.
10. Hinz, M., et al., *NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition.* Mol Cell Biol, 1999. **19**(4): p. 2690-8.
11. Sewify, E.M., et al., *Cyclin D1 amplification in multiple myeloma is associated with multidrug resistance expression.* Clin Lymphoma Myeloma Leuk, 2014. **14**(3): p. 215-22.
12. Narasimha, A.M., et al., *Cyclin D activates the Rb tumor suppressor by mono-phosphorylation.* Elife, 2014. **3**.
13. Diehl, J.A., et al., *Hsc70 regulates accumulation of cyclin D1 and cyclin D1-dependent protein kinase.* Mol Cell Biol, 2003. **23**(5): p. 1764-74.
14. Jirawatnotai, S., et al., *A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers.* Nature, 2011. **474**(7350): p. 230-4.
15. Bocker, W., *[WHO classification of breast tumors and tumors of the female genital organs: pathology and genetics].* Verh Dtsch Ges Pathol, 2002. **86**: p. 116-9.
16. Iida, M., et al., *Decreased ER dependency after acquired resistance to CDK4/6 inhibitors.* Breast Cancer, 2020. **27**(5): p. 963-972.
17. Yang, C., et al., *Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence.* Oncogene, 2017. **36**(16): p. 2255-2264.
18. Pestell, R.G., *New roles of cyclin D1.* Am J Pathol, 2013. **183**(1): p. 3-9.
19. Musgrove, E.A., et al., *Cyclin D as a therapeutic target in cancer.* Nat Rev Cancer, 2011. **11**(8): p. 558-72.
20. Licciardi, P.V. and T.C. Karagiannis, *Regulation of immune responses by histone deacetylase inhibitors.* ISRN Hematol, 2012. **2012**: p. 690901.
21. Moreno-Bueno, G., et al., *Cyclin D1 gene (CCND1) mutations in endometrial cancer.* Oncogene, 2003. **22**(38): p. 6115-8.
22. Gong, X., et al., *Genomic Aberrations that Activate D-type Cyclins Are Associated with Enhanced Sensitivity to the CDK4 and CDK6 Inhibitor Abemaciclib.* Cancer Cell, 2017. **32**(6): p. 761-776 e6.
23. Zhou, X., et al., *Inhibition of cyclin D1 expression by cyclin D1 shRNAs in human oral squamous cell carcinoma cells is associated with increased cisplatin chemosensitivity.* Int J Cancer, 2009. **124**(2): p. 483-9.
24. Kennedy, A.L., et al., *Complementary Targeting of Rb Phosphorylation and Growth in Cervical Cancer Cell Cultures and a Xenograft Mouse Model by SHetA2 and Palbociclib.* Cancers (Basel), 2020. **12**(5).