**RESEARCH WATCH**

## Leukemia

**Major finding:** The circadian rhythm transcription factors CLOCK and BMAL1 are essential for AML cell growth.

**Concept:** AML cells and HSCs have an intact circadian clock, but AML cells are more sensitive to disruption.

**Impact:** Targeting the circadian rhythm genes may reduce AML growth and promote differentiation.

**DISRUPTION OF CORE CIRCADIAN CLOCK GENES REDUCES LEUKEMIA GROWTH**

Acute myeloid leukemias (AML) often exhibit transcription factor dependencies that may drive leukemogenesis by preventing differentiation and generating leukemia stem cells that are essential for disease initiation and progression. Puram and colleagues performed an *in vivo* shRNA screen to identify leukemia-specific transcription factor dependencies in a serial transplantation model of AML driven by MLL–AF9, and found 35 transcriptional regulators that are likely essential for AML cells, including two circadian rhythm genes, *Clock* and *Bmal1*, which function as a heterodimer in circadian clock regulation. The circadian clock has known functions in the hematopoietic system and has previously been shown to regulate cellular proliferation, and thus may be a potential driver for cancer. Disruption of the circadian rhythm through inhibition of BMAL1 transcription with the small molecule SR9011 resulted in an increase in myeloid differentiation markers and a reduction in actively dividing cells. Further, murine AML cells and normal HSCs exhibited a functional circadian clock, as luciferase fused to the clock gene Per2 resulted in bioluminescence oscillations within a period of approximately 24 hours. In human AML cells CLOCK and BMAL1 heterodimers were enriched at genes involved in the circadian rhythm, suggesting a functional circadian clock. Moreover, human AML cell lines were sensitive to CLOCK and BMAL1 knockdown. Specific hematopoietic deletion of Bmal1 did not result in hematopoietic defects, indicating that Bmal1 loss is well tolerated in adult hematopoiesis and suggesting that clock gene inhibition may selectively target cancer cells. Conditional deletion of Bmal1 in murine MLL–AF9 leukemia resulted in a shortened survival, and AML cells were more sensitive to circadian disruption with SR9011 than normal hematopoietic cells. Together, these results indicate AML cell dependence on the circadian rhythm genes CLOCK and BMAL1, and suggest that the clock genes may be effective therapeutic targets in AML.


## Prostate Cancer

**Major finding:** Surgical castration synergizes with immunotherapy, but chemical castration has suppressive effects.

**Mechanism:** AR antagonists impair T cells in part through off-target effects on GABA-A receptor signaling.

**Impact:** Blocking androgen synthesis may synergize with immunotherapy and avoid off-target effects.

**ANDROGEN RECEPTOR ANTAGONISTS SUPPRESS IMMUNOTHERAPY IN PROSTATE CANCER**

Androgen deprivation therapy (ADT) is central to prostate cancer treatment and can be performed through surgical castration (orchietomy), or medically through combined androgen blockade (CAB) with luteinizing hormone-releasing hormone analog (LHRH-A) and androgen receptor (AR) antagonists. Orchietomy promotes antitumor immunity, and medical castration may as well; however, the effect of CAB on immune response remains controversial. Clinical trials evaluating immunotherapy with medical castration are under way, but it is not yet clear how immunotherapy can be combined with ADT. Pu and colleagues used the Myc-CaP transplantable prostate tumor model to study the effects of ADT on immune regulation. In this model, orchietomy induces rapid apoptosis and tumor antigen release, providing a potential immunotherapeutic window. Orchietomy synergized with CpG, a toll-like receptor agonist that activates dendritic cells, to reduce tumor volume, whereas CAB suppressed the CpG-induced immune response and resulted in earlier relapse. Moreover, the AR antagonist flutamide alone prevented the response to CpG in an androgen-independent manner. T-cell activation was reduced in mice treated with CpG and flutamide compared with CpG alone, indicating that AR antagonists can suppress T-cell activation, and suggesting that the efficacy of prostate cancer immunotherapy might be improved by CAB therapy following immune system activation. Nonsteroidal AR antagonists, including flutamide, engage the GABA-A receptor, which is expressed on immune cells and suppresses the immune response, suggesting that the immunosuppressive effects of AR antagonism may be mediated by off-target effects on GABA-A signaling. However, abiraterone, a steroidal inhibitor of androgen synthesis, avoided the off-target effects on GABA-A receptors and enhanced the effects of CpG, extending survival and leading to near-complete tumor regression. Together, these findings indicate that AR antagonists may potentially antagonize immunotherapy and suggest that inhibition of androgen synthesis may avoid the negative effects. Further, the timing and type of ADT may be critical for improving the efficacy of combination ADT/immunotherapy strategies in cancer.

Androgen Receptor Antagonists Suppress Immunotherapy in Prostate Cancer

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