

Androgen Deprivation Reprograms Human CD8⁺ T Cell Fate Through an Androgen Receptor–BHLHE40 Axis

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Background: Most non-reproductive cancers are male-biased in incidence and mortality. Our laboratory and others implicated androgen receptor (AR) signaling as a cell-intrinsic driver of CD8⁺ T cell exhaustion, but its human relevance and transcriptional effectors remain undefined. Androgen deprivation therapy (ADT) for men with prostate cancer (PrCA) offers a controlled setting to interrogate AR signaling in CD8⁺ T cells. BHLHE40 governs the progenitor-to-terminal exhaustion transition in murine CD8⁺ T cells (Wu et al., 2023) and is AR-repressed in prostate epithelium (Horestani et al., 2024). We hypothesized that AR sustains the progenitor-exhausted (Tpex) state by repressing *BHLHE40*, and that ADT reprograms CD8⁺ T cell fate via an AR–BHLHE40 axis.

Methods: In an IRB-approved study, peripheral blood mononuclear cells from 18 men with PrCA, collected before and 1, 3, 6, and 12 months after ADT, underwent high-dimensional flow cytometry. Single-cell RNA-sequencing (scRNA-seq) profiled enriched CD8⁺ T cells at baseline and 3 months in 4 men with metastatic PrCA. Six-marker multiplex immunofluorescence with spatial analysis compared prostatectomy specimens from 13 neoadjuvant-ADT-treated men and 13 matched controls. Mechanistic studies included CRISPR/Cas9 *BHLHE40* knockout (KO) in primary human and murine CD8⁺ T cells within in vitro exhaustion assays, murine DHT dose-response, murine AR CUT&TAG, and human AR ChIP-seq.

Results: Castration was biochemically confirmed (testosterone 357 to 12 ng/dL). ADT altered circulating CD8⁺ T cell differentiation time-dependently: at 1–3 months, CD45RA^{neg}TCF1^{high}PD-1^{int} Tpex cells decreased (14.2% to 8.1%, *p*=0.007), GZMB^{high} effector-like cells increased, and TOX^{high}PD-1^{high} terminally exhausted (Tex)-like cells decreased; by 6–12 months these shifts partially reverted and FOXP3⁺CD25⁺ regulatory T cells expanded. scRNA-seq identified *BHLHE40* as the second most upregulated gene within a proliferating Tpex-like cluster, alongside downregulated *TCF7* and a shifted AR-response program. Intratumorally, neoadjuvant ADT increased CD8⁺GZMB⁺ effector infiltration, decreased Tex frequency, raised the Tpex:Tex ratio, and increased Tpex–Tex spatial separation at early but not late timepoints; higher tumoral Tpex density was associated with improved overall survival (*p*=0.021). *BHLHE40* KO restricted activation and terminal exhaustion in human and murine CD8⁺ T cells while preserving naive states. DHT repressed *Bhlhe40* dose-dependently in murine CD8⁺ T cells, mirroring its de-repression after ADT. AR ChIP-seq confirmed AR binding upstream of *BHLHE40* in human cells; murine AR CUT&TAG showed AR occupancy at active-promoter chromatin.

Conclusions: These data provide temporally resolved human evidence that ADT reprograms CD8⁺ T cell fate, extending the CD8-intrinsic AR–exhaustion paradigm into humans and linking it to an AR-repressed effector, BHLHE40. An AR–TCF7/BHLHE40 axis thus forms a hormonally controlled network restraining CD8⁺ T cells in a Tpex state, released by ADT to drive progenitor-to-effector differentiation. Because early ADT engages the same axis as immune checkpoint blockade (ICB), these findings support ADT-ICB combination and nominate BHLHE40 as a therapeutic target.

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