**ABSTRACT**

**Background.** The Bromodomain and Extra-Terminal (BET) protein BRD4 is a key transcriptional regulator of various oncogenes (e.g., MYC) in numerous human malignancies and BET inhibitors have entered clinical trials. We have developed a proteolysis-targeting chimera (PROTAC) molecule, ARCC-29, which induces degradation of BRD4 in several human malignant cell lines and in-vivo xenograft tumors. In rodent xenograft models of prostate (22Rv1), DLBCL (U2932), and ovarian (A2780) tumors, this molecule demonstrates a strong antitumor efficacy. In clinical trials, acquiring tumor tissue from patients can be invasive and difficult, sometimes impossible. Looking ahead to clinical trials, we have targeted PBMC and skin as surrogate tissues to monitor PROTAC mode-of-action during clinical trials.

**Methods and Results.** We have developed a flow cytometry protocol to quantitatively assess BRD4 levels in human and rat peripheral blood mononuclear cells (PBMCs). Human (ex- and in-vivo) and rat (in-vivo) PBMCs treated with ARCC-29 were fixed and permeabilized for BRD4 staining. BRD4 was detected using anti-BRD4 antibodies in fresh and thawed PBMCs. Betel and RITA-015 showed similar or increased levels of BRD4 compared to vehicle controls, as did in vivo treatments (e.g., ARCC-29). Immunochemistry (IHC) can evaluate BRD4 degradation in skin biopsies of rats. BRD4 IHC following ARCC-29 treatment demonstrated a dose-dependent reduction in BRD4 protein.

**Conclusions.** BRD4 degradation can be evaluated in surrogate tissues. Flow cytometry using PBMCs and skin IHC provide alternative methods for confirming PROTAC mechanism-of-action in the clinic using pre- and post-treatment patient samples.

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**PROTAC: PROteolysis Targeting Chimera**

- Technology developed by Prof. Craig Crews, Yale University
- Platform licensed to Arvinas in 2013

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**A BRD4 FACS assay with fresh PBMCs**

- Depleted BRD4 can be reproducibly measured by fluorescence-activated cell sorting (FACS) in the lymphocyte subset of fresh human PBMC treated ex-vivo with ARCC-29 and the BETI GTX-015 (Fig. 3).

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**BRD4 FACS in frozen/thawed PBMCs**

- Depleted BRD4 can be reliably detected by FACS in ex-vivo ARCC-29 treated frozen/thawed lymphocyte subset of normal human PBMCs (Fig. 4).

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**In-vivo BRD4 depletion in rat PBMCs**

- Depletion of BRD4 can be reproducibly measured by FACS in the lymphocyte subset of nude rat PBMCs dosed ex-vivo with ARCC-29 (Fig. 6).

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**Skin BRD4 depletion evaluation by IHC**

- Nude rat skin biopsies show a dose-dependent depletion of BRD4 in-vivo following 2 and 0.3 mg/kg (ip) IV, 5 hours post-dose (Fig. 7). A2780 (ovarian cancer) human xenograft tumors used as controls. Figure 8 shows example staining in normal untreated human skin.

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**Conclusion**

We have developed two assays to monitor BET PROTAC mode-of-action in clinical trials using PBMCs and skin as surrogate tissues for tumor. PBMCs and skin biopsies are easily acquired, stored and transported for analysis.

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