

ABSTRACT

Background. The Bromodomain and Extra-Terminal (BET) protein BRD4 is a key transcriptional regulator of various oncogenes (eg MYC) in numerous human malignancies and BET inhibitors have entered clinical trials. We have developed a proteolysis-targeting chimera (PROTAC) molecule, ARCC-29, which degrades BET (BRD4/3/2) proteins in several human malignant cell lines and in *in-vivo* rodent xenograft tumors. In rodent xenograft models of prostate (ZZRv1), DLBCL (SUJHL-6) and ovarian (A2780) tumors, this molecule demonstrates a strong PK/PD/efficacy relationship. In clinical trials, acquiring tumor tissue from patients can be invasive and/or impossible. Looking ahead to the future, we have developed a PROTAC mode-of-action during clinical trials.

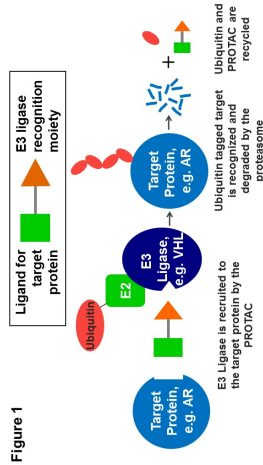
Methods and Results. We have developed a flow cytometry protocol to quantitatively assess BRD4 levels in human and rat peripheral blood mononuclear cells (PBMCs). Human (ex-vivo) and rat (in-vivo) PBMCs treated with ARCC-29, were fixed and permeabilized for BRD4 staining. ARCC-29 dramatically reduced BRD4 levels in fresh and thawed PBMCs. BET inhibitor OTX-015 showed similar or increased levels of BRD4 compared to vehicle controls, as did inactive stereoisomers of ARCC-29. Immunohistochemistry (IHC) can evaluate BRD4 degradation in skin biopsies of rats. BRD4 IHC following ARCC-29 treatment demonstrated a dose-dependent reduction of BRD4 protein.

Conclusions. BRD4 degradation can be evaluated in surrogate tissue from patients and in PBMCs. This is the first time that a PROTAC has been used to evaluate the mechanism-of-action in the clinic using pre- and post-treatment patient samples.

PROTAC: PROteolysis Targeting Chimera

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

Figure 1

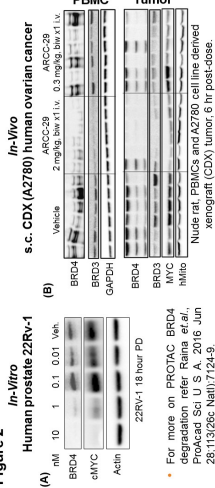


Selected publications on PROTAC technology:
 • PNAS 2016 Jun 28;113(26):7124-9
 • Nat Chem Biol. 2015 Jun 1;11(6):111-7
 • Nat Reviews Drug Discov. 2017 Feb; 16(2):101-114.

BET PROTAC DEGRADES BRD4

- ARCC-29 PROTAC degrades BRD4 and BRD3 in rat PBMCs *in-vivo*, a viable surrogate tissue for tumor to monitor drug mode-of-action in clinical trials (Fig 2).

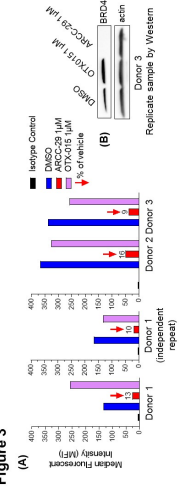
Figure 2



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 BET1 OTX-015 (Fig 3).

Figure 3



FACS Materials and Methods

- Human PBMCs dosed *ex-vivo* for 6 hours, fixed & permeabilized (TF Buffer, Set BD Biosciences). Blocked with human AB serum (ZenBio).
- Primary anti-BRD4 (rabbit HPA015055, Sigma) + 2° goat anti-rabbit-AF488 (Abcam, ab150077).
- Isozyme - primary rabbit IgG (ab119009) + 2° anti-rabbit-AF488 (ab150077).
- Western - ab HPA015055 (Sigma) + 2° HRP-goat anti-rabbit.
- Gated lymphocyte BRD4 levels are shown (FACScellular).

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 (ZenBio) (Fig 4, 5).

Figure 4

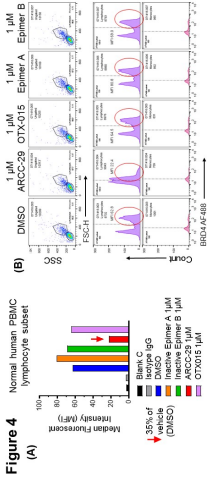
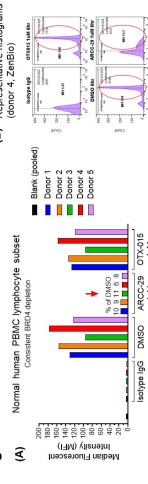


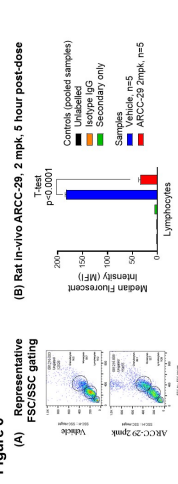
Figure 5



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 *in-vivo* with ARCC-29 (Fig 6).

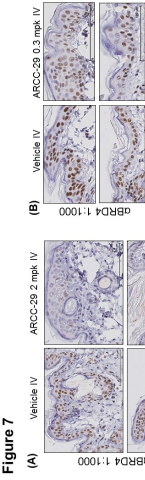
Figure 6



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 (mpk) 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.

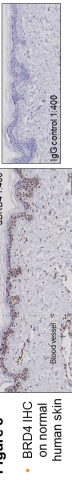
Figure 7



IHC Materials & Methods

- 10% neutral buffered formalin fixed, paraffin-embedded, 5-µm tissue sections.
- 1° anti-BRD4 antibody (Gephyr Labs A301-985A), 2° biotinylated goat anti-rabbit + streptavidin-HRP conjugate.
- Ventana XT instrument, Ventana reagent and detection kits.

Figure 8



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.

Acknowledgements

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