Exploiting the Potential of Bromodomain Inhibitors in Ewing Sarcoma

Shireen Ganapathi MD[1,2], Nicolas Garcia[1], Taran S. Gujral PhD[3,4], Elizabeth R. Lawlor MD, PhD [1,2,3]

University of Washington Department of Pediatrics, Division of Pediatric Hematology/Oncology[1], Seattle Children's Research Institute[2] Fred Hutchinson Cancer Research Center, Human Biology[3] University of Washington Department of Pharmacology [4]

Background

- Ewing sarcoma (ES) is a bone and soft tissue tumor that most commonly occurs in the adolescent and young adult population.
- Survival rates for metastatic and relapsed disease are dismal (<25%) with therapies unchanged for the last 30 yrs. [1-3]
- Defined by the tumor initiating fusion, most common EWS-FLI1 (~85%) which causes global transcriptional dysregulation. [4,5]
- Bromodomain and extraterminal domain (BET) proteins are epigenetic readers that recognize acetylated histone residues and facilitate transcription. [6]
- BET inhibitors effectively block transcription at superenhancers, enhancers, and promoters, but not curative as a single agent. [6,7]
- BMS-986158 is the first BET inhibitor in pediatrics as part of an early phase clinical trial for relapsed solid, brain, and lymphomas (NCT03936465).

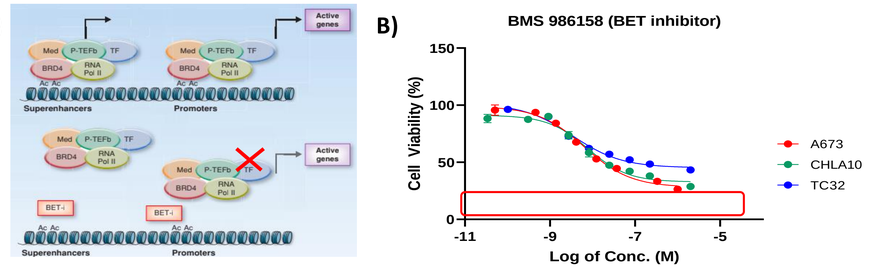

Figure 1. Epigenetic therapies in Ewing sarcoma A) BET inhibitors are small molecule inhibitors that bind within the pocket that BET proteins would normally bind to acetylated histone marks. This prevents binding and recruitment of transcriptional machinery. JQ1, the first BET inhibitor discovered, showed to effectively downregulate the oncogene *myc.*[6] **B)** BMS-986158 has a low nanomolar IC50 in multiple ES cell lines. However, the highlighted red box indicates the surviving population of cells. The success of BET inhibitors is identifying escape mechanisms and targeting those in combination.

Objectives: 1) Identify combinatorial drug strategies with BMS-986158 2) Identify mechanisms of escape with BMS-986158 in ES

Single agent BMS-986158 in ES

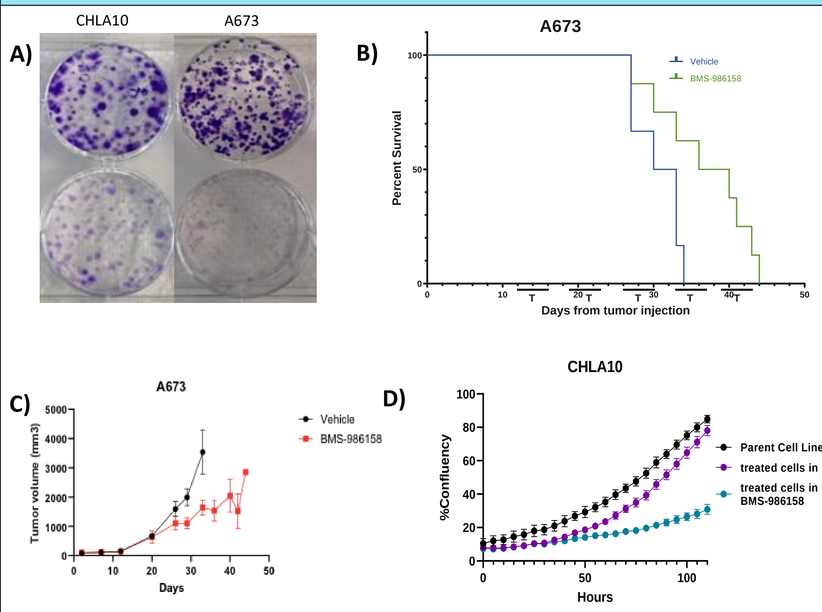

Figure 2. BMS-986158 single agent leads to slowed growth *in vitro* and *in vivo.* A) Colony formation assay of ES cells growing in standard condition media (top) and with added IC50 dose of BMS-986158 (bottom). A673 subcutaneous xenograft mouse model treated with BMS-986158 reveals **B)** increased survival and **C)** slowed tumor growth. **D)** Real time proliferation assay (Incucyte) of CHLA10 cells treated in culture for 20 days with IC50 equivalent BMS-986158. Continued drug treatment shows sustained slowed proliferation. With removal of drug, there is quick rebound back to proliferation rates of parent cell line.

BMS-986158 single agent does not lead to tumor regression *in vivo* and proliferation is reversible *in vitro* upon removal of treatment. Thus, the need to identify combinatorial strategies

BET inhibition combined with PI3K inhibition in synergistic *in vivo*

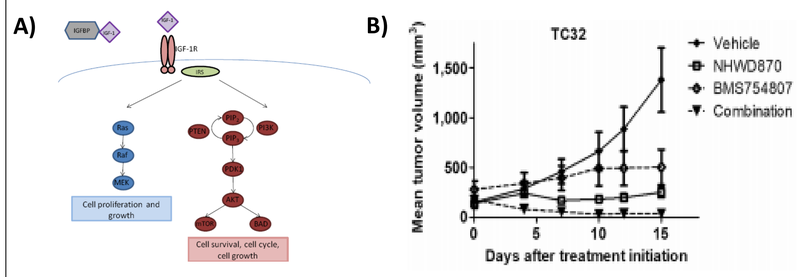

Figure 3. Targeting the IGF1R/PI3K/AKT pathway with BET inhibition. A) IGF1R pathway is a well established oncogenic pathway in ES [8]. **B)** The IGF1R/PI3K/AKT pathway is an established pathway of resistance with BET inhibition in neuroblastoma and ES [9,10]. Shown is tumor regression in a subcutaneous ES xenograft model receiving combination therapy of BET inhibition (NHWD870) with IGF1R inhibition (BMS754807) [10]. **C)** Cell viability assay of single agent PI3K inhibitor Copanlisib with nanomolar IC50 doses. Copanlisib is part of an early phase clinical trial in pediatrics for relapsed solid tumors (NCT03458728).

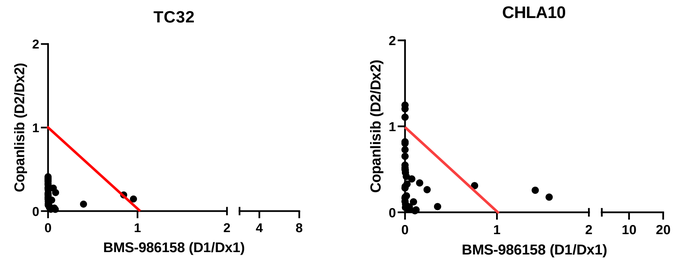

Figure 4. BMS-986158 and Copanlisib combination are synergistic *in vitro*. Normalized isobolograms depicting the combination index (CI) scores over a range of concentrations. The coordinates of the CI scores are D1/Dx1 and D2/Dx2, where D1 (BMS-986158) and D2 (Copanlisib) are the dose of drug 1 (BMS-986158) and drug 2 (Copanlisib) alone to generate the same effect X. The data falling in the triangle, depicting CI values <1, indicate synergism, points on the red line, CI value =1, are additive, and above the line in the open area, CI >1 indicate antagonism.

Future Directions: Test BMS-986158 + Copanlisib *in vivo*

BET inhibition leads to transcriptional rewiring and suggests epigenetic rewiring

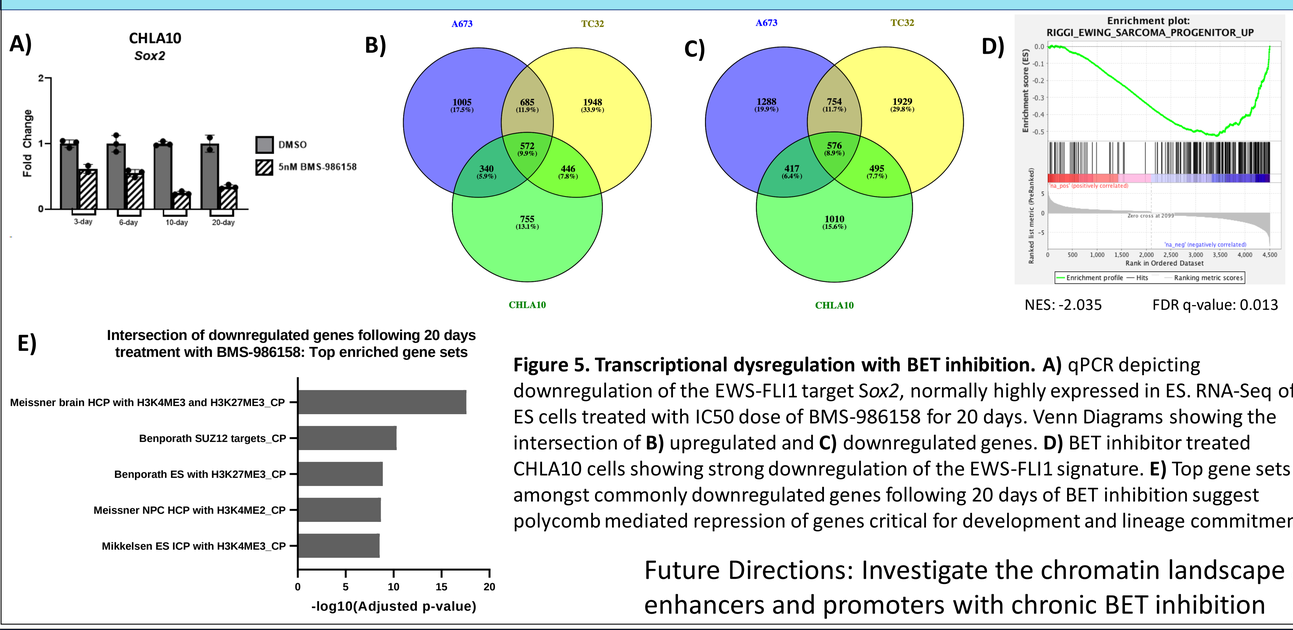

Figure 5. Transcriptional dysregulation with BET inhibition. A) qPCR depicting downregulation of the EWS-FLI1 target S*ox2*, normally highly expressed in ES. RNA-Seq of ES cells treated with IC50 dose of BMS-986158 for 20 days. Venn Diagrams showing the intersection of **B)** upregulated and **C)** downregulated genes. **D)** BET inhibitor treated CHLA10 cells showing strong downregulation of the EWS-FLI1 signature. **E)** Top gene sets amongst commonly downregulated genes following 20 days of BET inhibition suggest polycomb mediated repression of genes critical for development and lineage commitment.

Future Directions: Investigate the chromatin landscape at enhancers and promoters with chronic BET inhibition

Identifying and targeting additional dysregulated kinase pathways with BET inhibition

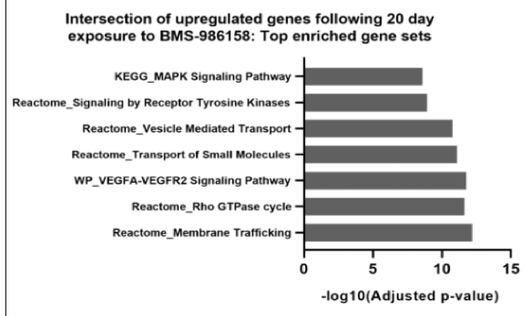

Figure 6. Chronic treatment with BMS-986158 suggests augmentation of kinase and cell signaling pathways. Top gene sets amongst commonly upregulated genes following 20 days of BET inhibition. Multiple gene sets critical for cell growth, signaling, and survival were enriched, suggesting additional pathways of escape.

Future Directions: Collaboration with the Gujral Laboratory- kinase inhibitor screen[11,12] to identify and target dysregulated pathways with BET inhibition

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