ACVR1-Mutant DIPGs are Characterized By Aberrant Activin-Mediated BMP Signaling but May Lack Cytotoxic Response to Targeted ACVR1 Receptor Inhibition

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Disclosures

• No relevant conflicts of interest
Introduction

- ACVR1 mutations are found in 20-25% of diffuse intrinsic pontine gliomas (DIPGs; Fig. 1)\(^1\)
- ACVR1 is a Type 1 Bone Morphogenetic Protein (BMP) receptor, transmits mitogenic signal in response to BMP
- ACVR1 mutations also underlie fibrodysplasia ossificans progressiva (FOP): pathologic bone overgrowth\(^2\)
  - Aberrant ACVR1 activation occurs in response to the non-canonical ligand activin (Fig. 2)
  - Inhibiting ACVR1 reduces pathologic bone formation in FOP
- Study goal: evaluate whether this aberrant activin signaling also occurs in DIPGs and assess the impact of ACVR1-specific inhibitors on cell growth and viability as a potential targeted therapy

\(^1\) Han H, et al. Bone. 2017
\(^2\) Hatsel, et al. Sci Trans Med. 2015
Methods

- Patient-derived mutant ACVR1 (ACVR1\textsuperscript{G328V}) and wild-type (WT) DIPG cell lines were assessed by Western blot and RT-PCR for their response to a member of the activin family, Activin A, and BMP
- Overexpression of ACVR1\textsuperscript{G328V} and WT ACVR1 in HEK293T cells was used to further characterize the BMP signaling response to Activin A or BMP
- Patient-derived lines were exposed to multiple ACVR1-targeted inhibitors. Cell viability assays were performed alongside Western blot analysis for BMP signaling
- RNA-Seq was performed on patient-derived cell lines exposed to BMP and Activin A for identification of other contributing signaling pathways
Results: ACVR1 mutant DIPG acquires aberrant response to Activin A

Figure 3. A) Patient-derived DIPG cell lines and their associated mutations. B) ACVR1\textsuperscript{G328V} DIPG (DIPG IV) acquires aberrant SMAD 1/5/9-dependent BMP signaling to Activin A (red box). C) BMP pathway target genes (Id1, Id2, Id3) show aberrant upregulation in response to Activin A exposure only in ACVR1\textsuperscript{G328V} DIPG cell lines assessed by RT-PCR.

<table>
<thead>
<tr>
<th>Patient-derived DIPG cell lines</th>
<th>Mutation</th>
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<tbody>
<tr>
<td>JHH-DIPG1</td>
<td>H3F3A K27M, ACVR1 WT</td>
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<tr>
<td>SU-DIPG IV</td>
<td>HIST1H3B K27M, ACVR1 G328V</td>
</tr>
<tr>
<td>SU-DIPG XIII</td>
<td>H3F3A K27M, ACVR1 WT</td>
</tr>
<tr>
<td>SU-DIPG XVII</td>
<td>H3F3A K27M, ACVR1 Wt</td>
</tr>
<tr>
<td>SU-DIPG VI</td>
<td>H3F3A K27M, ACVR1 WT</td>
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A

<table>
<thead>
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<tr>
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B

- p-SMAD 1/5/9
- p-SMAD 2/3
- t-SMAD 5
- t-SMAD 2/3
- beta-actin

C

- Id1
- Id2
- Id3

<table>
<thead>
<tr>
<th>Relative Quantity (normalized to GAPDH)</th>
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<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>Id1</td>
</tr>
<tr>
<td>Id2</td>
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<td>Id3</td>
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</tbody>
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Results: Overexpression of various ACVR1 mutants confers aberrant response to Activin A

Figure 4. A) Overexpression in HEK293Ts of wild-type and various mutant ACVR1 found in DIPG samples shows aberrant activation of SMAD 1/5/9-dependent BMP signaling on exposure to Activin A (red box). B) Luciferase reporter assay linked to BMP signaling in HEK293T also showed aberrant activation in response to Activin A when various mutant ACVR1 are overexpressed.
Results: Activin A inhibits *in vitro* DIPG growth; ACVR1-specific inhibitors lack significant cytotoxicity *in vitro*

Figure 5. Treatment with Activin A (red) and BMP (blue) showed variable decreases in DIPG cell line growth *in vitro* regardless of ACVR1 mutational status.

Figure 6. A) Experimental timeline. B) Treatment with non-specific ACVR1 inhibitor LDN193189 showed expected *in vitro* killing across cell lines, but it has significant off-target effects. C) ACVR1-specific inhibitors showed minimal effect on cell viability, independent of ACVR1 mutational status. Western blots (not shown) revealed inhibition of BMP pathway by all of these compounds despite lack of toxicity.
Results: RNA-Seq suggests role for alternative signaling pathways in ACVR1\textsuperscript{G328V} DIPG

- Treatment of two WT and one ACVR1\textsuperscript{G328V} patient-derived DIPG cell lines with Activin A or BMP
  - RNA-seq with gene set variation analysis performed: method for determination of gene expression alterations across pathways
- 449 pathways upregulated and 348 downregulated in ACVR1\textsuperscript{G328V} cell line treated with Activin A
  - Significantly more than in BMP treated samples (36 and 64, respectively)
- Genes frequently involved in upregulated pathways in Activin A treated ACVR1\textsuperscript{G328V} cell line:
  - Notch
  - NKK1 (regulator of WNT signaling)
Discussion

• ACVR1-mutant DIPGs respond aberrantly to Activin A, activating SMAD-dependent BMP pathway target genes

• Exposure to Activin A in vitro inhibits DIPG growth, regardless of ACVR1 mutational status
  • Activin shown to be tumor suppressive as well as tumorigenic¹

• ACVR1-specific inhibition does not seem have significant cytotoxic effect on in vitro DIPG growth
  • No specific effects in ACVR1-mutant cells
  • Cytotoxic results² seen previously with ACVR1 inhibitor (LDN193189) may be more attributable to off target effects (FGFR1, RIPK2 → MAPK pathway)

• Gene set variation analysis suggests rationale for targeting Notch and WNT pathways
  • Supported by previous studies showing effect of Notch inhibition³

Summary

• ACVR1-mutant DIPGs acquire aberrant response to the non-canonical ligand activin

• Activin exposure *in vitro* did not promote growth in WT or mutant DIPG

• Non-specific ACVR1 inhibitor recapitulates observed cytotoxicity but specific ACVR1 inhibition had no consistent effect on cell viability in WT and ACVR1 mutant cell lines
  • Questions role for ACVR1 inhibition as monotherapy

• Role of Notch and WNT signaling under further investigation

• CRISPR/Cas9 screen to find other essential pathway nodes is being performed