Systemic Targeting of a CNS tumor (Medulloblastoma) using a novel cell-penetrating, nucleic acid binding, monoclonal antibody

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Poster: P153
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I have the following financial relationships to disclose:

Consultant for: Gennao Bio
Stockholder in: Gennao Bio
Overview

- Background on 3E10
- Characterization of nucleic acid binding
- Biodistribution of 3E10 in an orthotopic CNS tumor model
- Application: delivery of immunostimulatory RNA to trigger an anti-tumor response
Identification of 3E10, a DNA-binding, cell-penetrating antibody

Mouse model of Lupus (MRL-lpr/lpr)

Isolate Antibodies

Screen Binding to dsDNA

Immunize Mice

Mouse model of Lupus (MRL-lpr/lpr)

3E10 penetrates cells through a nucleoside transporter, ENT2, and requires extracellular nucleic acid.

A. PK15NTD/ENT1 Cells

B. PK15NTD/ENT2 Cells

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Can 3E10 (GMAB) be used to deliver nucleic acids to cells?
GMAB variant, D31N demonstrates superior binding to DNA relative to parental wild-type.
GMAB delivers nucleic acids to cells *in vitro*

Labeled Oligo

GMAB/Oligo Complex
Evaluating GMAB targeting and delivery to target a human CNS tumor (Medulloblastoma; DAOY)

Orthotopic Model of a Human Medulloblastoma (DAOY)
Evaluating GMAB targeting and delivery to a human CNS tumor (Medulloblastoma; DAOY)

Two Routes of Administration

Intracisternal

Intravenous

A

B

Cisterna Magna

Occipital crest

Cannula with CSF tracer


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Intracisternal injection of labeled-GMAB demonstrates sustained tumor retention.
Intravenous injection of labeled-GMAB demonstrates its ability to penetrate the CNS and localize to tumors.
Triggering an anti-tumor response by stimulating RIG-I

Detection of 5' triphosphate RNA by RIG-I

Innate Immune System
Type-I IFN Response

Tumor Cells
Immunogenic Cell Death

Potent anti-tumor response
GMAB delivery of 3p-hpRNA, a RIG-I agonist, elicits a type-I IFN response

*In vitro* delivery of 3p-hpRNA to THP-1 monocytes

**Immune Stimulation by dsRNA**

<table>
<thead>
<tr>
<th></th>
<th>Type 1 IFN Response (fold increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0</td>
</tr>
<tr>
<td>3p-hpRNA</td>
<td>~20</td>
</tr>
<tr>
<td>GMAB</td>
<td>~60</td>
</tr>
<tr>
<td>GMAB-3pRNA</td>
<td>~80</td>
</tr>
</tbody>
</table>

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AACR-NCI-EORTC VIRTUAL INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS
Intravenous injection of GMAB/3p-hpRNA leads to a significant reduction in tumor burden.
Intravenous injection of GMAB/3p-hpRNA leads to prevention of spinal metastasis

• A single dose of GMAB\textsuperscript{D31N}/3p-hpRNA significantly reduces tumor burden

• A single dose of GMAB\textsuperscript{D31N}/3p-hpRNA significantly prevents spinal metastasis
GMAB is a DNA-dependent, cell-penetrating antibody, which enters cells via ENT2

GMABs bind directly to DNA and RNA, with higher affinity observed for the D31N variant

GMABs can deliver nucleic acids directly into cells without chemical linkers through non-covalent interactions, mapped to the CDRs (not shown)

GMABs localize to sites of high ENT2 expression including tumors

In a mouse model of medulloblastoma, intravenous administration of GMAB/3p-hpRNA complexes resulted in a significant reduction in tumor burden and prevention of spinal metastasis

For further discussion, please contact Elias.Quijano@Yale.edu or Peter.Glazer@Yale.edu