CNS PENETRANT AAV VECTORS ENCODING HER2 ANTIBODIES REDUCE TUMOR BURDEN IN MODELS OF BREAST CANCER BRAIN METASTASIS

ASGCT 25TH ANNUAL MEETING

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Rm 204, Abstract #63
May 16, 2022, 5:15 pm
Dan Laks is a full-time employee of Voyager Therapeutics
Hypothesis: AAV mediated production of anti-HER2 antibodies in the CNS will mitigate prognosis of metastatic HER2+ breast cancer

Addressing an unmet medical need

AAV gene therapy can enter the brain and generate stable expression of therapeutic antibodies

Systemic αHER2 antibody therapies are limited in their CNS distribution by the blood-brain barrier and CNS efflux

Conventional Antibody

rAAV
Strategy: Employ brain cells as antibody factories through AAV mediated gene therapy

Vectorized antibodies are a method to generate persistent, high coverage target-engagement in the CNS
Transgene: A vectorized αHER2 mAb with enhanced ADCC (ADCC+) properties

**Cell/Tumor Models**

**BT-474-Luc**: Primary HER2 ductal carcinoma, breast cancer tumor cells

**MDA-MB-361-Luc**: Originated from breast cancer brain metastasis tumor cells, of HER2 adenocarcinoma (glandular), cultured as tumorspheres
Proof of Concept Delivery: Broad expression in the brain using the Voyager TRACER Capsid 9P39

Sagittal mouse brain slices after i.v. administered AAV with 4e11 VG TRACER capsid 9P39, expressing EGFP transgene

9P39 has mouse specific BBB penetrance- serves as proof of concept

-9P39 data outlined in Nonnenmacher, 2020

IHC performed with anti-GFP antibody, a red chromogenic dye

5mm
HER2+ orthotopic xenograft brain tumors in mouse co-stain with vectorized αHER2 ADCC+ mAb

- HER2 Staining
- Human IgG Staining

% Tumor Area Stained with IgG1

P=0.0163

- Katy Tyson
HALO Analysis
Proof-of-principle: Demonstration of pre-clinical efficacy in 3 models

Model 1
Systemic Prevention of Metastasis

- i.v. administration of 5e11 Vg AAV-9P39 in a tumor prophylaxis study design
- CNS-targeted AAV vector
- Deliver AAV 12 days prior to engrafting MDA-MB-361-Luc tumorspheres (orthotopic)
- N=10 per group

Model 2
Systemic Treatment of Metastasis

- i.v. administration of 2.5e11 VG AAV-9P39 in a tumor treatment study design
- CNS-targeted AAV vector
- Deliver AAV 2 days post engrafting MDA-MB-361-Luc tumorspheres (orthotopic)
- N=5 per group

Model 3
Local Treatment of Metastasis

- Intra-tumoral administration of 6e10 VG AAV PHP.eB in a tumor treatment study design.
- Deliver AAV 2 days post engrafting BT-474-Luc cells.
- N=15 per group
vHER2 gene therapy confers survival benefit

**Model 2**

i.v. Administration in a Tumor Treatment Study Design (Mouse Orthotopic Xenograft)

- AAV-9P39 delivery 2 Days post xenograft of MDA-MB-361-Luc tumorsphere cells
- N=5 per group

**Median Survival**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days Post Xenograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>αHER2 mAb ADCC+ Isotype Ctrl</td>
<td>129.0</td>
</tr>
<tr>
<td>Isotype Ctrl</td>
<td>94.00</td>
</tr>
</tbody>
</table>

**Model 3**

Direct Intracranial Injection Administration in a Tumor Treatment Study Design (Mouse Xenograft)

- AAV PHP.eB delivery 2 Days post xenograft of BT474-Luc cells
- N=15 per group

**Median Survival**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days Post Xenograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>αHER2 mAb ADCC+ Isotype Ctrl</td>
<td>147.0</td>
</tr>
<tr>
<td>Isotype Ctrl</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Probability of Survival

- Days Post Xenograft
- Median Survival
- P-value

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Mechanism of Action scRNA-Seq Study Design

- **5x 9P39 Ctrl**
- **2.5e11 VG**
- **5x 9P39 αHER2 mAb ADCC+**

- **10X scRNA-Seq**
  - FACS for CD45 stained Viable (7AAD-) immune cells
  - Myelin Depletion
  - Peritumoral Mouse brain dissociation protease (cold)

- **Perfusion**
  - Hemi Brain, Tumor Side and area

- **28 Days total**
Mechanism of Action: Peritumoral CD45+ Immune Cells Demonstrate Differential scRNA-Seq Cluster Distribution from 9P39 mediated αHER2 mAb ADCC+ as compared to isotype control.

- Xiaoqin Ren (NGS)
Innate immune response is evident in αHER2 mAb ADCC+ treated cohort

CD45 Sorted Mouse Peritumoral Cells

<table>
<thead>
<tr>
<th>Cell Type (Cluster)</th>
<th>Microglia</th>
<th>cDC2</th>
<th>Macrophages</th>
<th>NK</th>
<th>ncMC</th>
<th>ILC2</th>
<th>pDC</th>
<th>migDC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conventional dendritic cells</td>
<td>Natural Killer</td>
<td>non classical monocytes</td>
<td>innate lymphoid cell</td>
<td>plasmacytoid dendritic cells</td>
<td>migratory dendritic cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Microglia State**
  - Normalized Proportion of Total Microglia Cells
  - Isotype Ctrl
  - αHER2 mAb-H10 ADCC+ /Isotype Ctrl

- **Normalized Proportion of Total Microglia Cells**
  - pMic

- **pMic** = proliferation microglia (Cluster 9)

Normalized to Isotype control
VCAP-102, a translational capsid that targets the CNS, identified by TRACER

VCAP-102 yielded a translatable enrichment over AAV9 in brain that was consistent between NHP and mouse.

Session Date/Time: Thursday May 19, 2022 10:15 AM - 12:00 PM
Session title: Novel AAV Capsids for the Brain, Eye and Kidney
Room: Ballroom A
Your Presentation Time: 11:00am - 11:15am
Final abstract number: 1198

Brain Expression

- Mathieu Nonnenmacher
- Tyler Moyer
Pre-clinical efficacy of VCAP-102-αHER2 mAb ADCC+ in prophylaxis treatment model

i.v. 2.5e11 VG VCAP-102 Prophylaxis Model Orthotopic Xenograft Treatment (MDA-MB-361L Cells)

- **Isotype Ctrl (Fc Modified)**
- **αHER2 mAb-H75 ADCC**

**N=5; 2 Way Anova Sidak’s multiple comparison test**

5x Difference in Tumor Burden at Day 41 Post Xeno
3x at Day 48

Day 53: VCAP-102 CSF vAb

- Established Efficacy

Day 53: VCAP-102 Vector Genome Copies: Brain

- 4.5
- 4.8

- Ishan Shah
- Jeff Thompson
- Joe Clement
A BBB penetrant capsid mediating vHER2 ADCC+ mAb brain expression represents a novel, single dose, systemic therapy for the treatment of HER2+ metastatic brain cancer

- Paired a functional ADCC+ vectorized antibody with CNS penetrant AAV including a translational capsid, VCAP-102
- This approach produced successful pre-clinical interventions that attenuated tumor burden and extended survival in mouse orthotopic xenograft models of metastatic brain cancer
- Potential mechanisms of action for our vHER2 gene therapy include inhibition of cell proliferation, ADCC and the innate immune response: Dendritic cells mediate between innate and adaptive immune responses

We plan to advance this gene therapy to tolerability and biodistribution studies in NHP
Acknowledgments

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Kenny Chen      Omar Bermudez
Usman Hameedi   Jiangyu Li
Kelly Bales     Ambreen Sayed-Zahid
Charlotte Chung Tyler Moyer
Maneesha Paranjpe Michael Grannan
THANK YOU FOR YOUR TIME

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