ENGINEERING CYTOLYTIC EFFECTOR CELLS FOR GLIOMA IMMUNOTHERAPY USING GENE INSERTION AND ZINC FINGER NUCLEASE GENOMIC EDITING

22ND ANNUAL iSBTc MEETING
BOSTON

DIVISION OF CANCER IMMUNOTHERAPEUTICS & TUMOR IMMUNOLOGY
BECKMAN RESEARCH INSTITUTE
TARGET POPULATION:

- Median survival duration following GBM relapse is 36-weeks

SEER DATA REPORTED BY DAVIS et al
GLIOMA TROPISM OF ADOPTIVELY TRANSFERRED EX VIVO PROPAGATED T-CELLS:

INTRAPARECHYMAL HOMING OF T-CELLS TO ORTHOTOPIC CNS GLIOMA XENOGRAFTS

T-CELLS USE WHITE MATTER TRACKS
PATIENT-DERIVED RESECTION CAVITY CSF CHEMOKINE CONTENT AND CHEMOTACTIC BIOACTIVITY

% Chemotaxis

Glioma CSF

Hydrocephalus CSF

Cytokine [pg/ml]

IL-8

IP-10

MCP-1

MIG

RANTES

Cytokine [pg/ml]

0 2500 5000 7500 10000 12500

TNF-α  MCP-1  IL-2  Pos
TNF-β  MCP-2  IL-3  Pos
EGF  MCP-3  IL-4  Neg
IGF-I  M-CSF  IL-5  Neg
Ang  MDC  IL-6  ENA-78
OSM  MIG  IL-7  GCSF
Tpo  MIP-1α  IL-8  GM-CSF
VEGF  RANTES  IL-10  GRO
PDGF-β  SCF  IL-12  GRO-α
Leptin  SDF-1α  IL-13  I-309
Neg  TARC  IL-15  IL-1a
Pos  TGF-β  IFN-γ  IL-1b

Pos  Neg
GLIOMA-DERIVED CCL2/MCP-1 DRIVES T-CELL TUMOR TROPISM

MCP-1 Quantitation
(CBA Analysis)

Chemotaxis Assay

- CM
- CM + anti-MCP-1
- CM + anti-IL-8
- CM + anti-MCP-1 + rhMCP-1
TUMOR SECRETED MCP-1 IS SUFFICIENT FOR *IN VIVO* HOMING OF ADOPTIVELY TRANSFERRED T-CELLS

**DAUDI-p**  **DAUDI-MCP1**

![Images showing cell infiltration]

**Quantification of T-cell Infiltration**

<table>
<thead>
<tr>
<th></th>
<th>T-cells per mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daudi-Parental</td>
<td>0.5</td>
</tr>
<tr>
<td>Daudi-MCP1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Intracranial Tumor Group**
TUMOR HOMING CYTOLYTIC T-CELLS:
NANOSURGEONS IN THE BRAIN

CYTOTOXIC T-LYMPHOCYTE:
A specialized white blood cell responsible for eliminating unwanted body cells (e.g. cancer) is killing a cell infected with the influenza virus.
EQUIPPING CTLs FOR TUMOR RECOGNITION BY GENE INSERTION
**SELECTION OF A GLIOMA-SPECIFIC TARGET ANTIGEN**

<table>
<thead>
<tr>
<th>TARGET:</th>
<th>NORMAL BRAIN</th>
<th>GLIOMA</th>
<th>T-CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnfR</td>
<td>+/-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>EGFR</td>
<td>++</td>
<td>++++</td>
<td>-</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>-</td>
<td>++++ (&lt;30%)</td>
<td>-</td>
</tr>
<tr>
<td>IL-13Rα2</td>
<td>-</td>
<td>++++ (&gt;90%)</td>
<td>-</td>
</tr>
</tbody>
</table>
THE IL13-ZETAKINE CHIMERIC ANTIGEN RECEPTOR FOR RE-DIRECTING CTL EFFECTOR FUNCTION TO GBM

IL13-ZETAKINE CHIMERIC ANTIGEN RECEPTOR

- huIL-13(E13Y)
- hulg4 hinge-Fc
- huCD3-ζcyto
- huCD4_{TM}

RE-DIRECTED HLA UNRESTRICTED ZETAKINE-REGULATED GLIOMA KILLING

[Image of a diagram illustrating the structure of the IL13-ZETAKINE CHIMERIC ANTIGEN RECEPTOR]
U87 GLIOBLASTOMA (ffLucZeo/IL-2)

DAY 0: $2 \times 10^5$ tumor cells i.c.

AntiCD19-CAR$^+$ CD8$^+$ CTL Clone E8

DAY +5: $10 \times 10^5$ CD8$^+$ CTL i.c.

IL13-zetakine$^+$ CD8$^+$ CTL Clone 2D7
GLIOMA STEM-LIKE TUMOR PROGENITOR CELLS

- CD133
- SOCS2
- EGF/FGF/LIF/NO SERUM
- DMEM 10%FCS
- CD133+/GFAP-
- CD133/GFAP+
PROTOCOL 01020 UPN-033: TARGET VALIDATION/T-CELL KILLING OF AUTOLOGOUS TUMOR/TUMOR STEM CELLS

GFAP⁺/CD133⁻ DIFFERENTIATED TUMOR

GFAP⁻/CD133⁺ TUMOR PROGENITORS

UPN033-IL13z @ S8D13

% Specific Lysis

E:T ratio

- PBT015 NS
- PBT015 Adh
- Daudi

IL13Ra2

PBT015 NS

PBT015 Adh

Daudi
CLINICAL APPLICATIONS
PLASMID EXPRESSION VECTOR
IL13 ZETAKINE/HyTK-pMG

IL13 Zetakine/HyTK-pMG

6785 bp
SCHEMA FOR T-CELL GENETIC MODIFICATION, CLONING, AND EX VIVO EXPANSION

Day 0
Polyclonal Activation of T Cells with OKT3

Day 3
Electroporation of OKT3-Activated PBMC

Day 5
Addition of Selection Drug

Day 28
Cloning of Surviving T Cells

Day 42
Expansion of Drug-Resistant Clones

Day 56
Large Scale Expansion of Zetakine+ Clones for Re-Infusion
PROCESS DEVELOPMENT

T-CELL BIOREACTORS
CLOSED SYSTEM CELL PROCESSING
IMMUNOMAGNETIC SELECTION
ENGINEERED FEEDER CELLS
OPTIMIZED CYTOKINE COMBINATIONS
THE CENTER FOR BIOMEDICINE AND GENETICS AT CITY OF HOPE

cGMP-MANUFACTURING FACILITY:

PLASMID DNA,
VIRAL VECTORS,
MONOCLONAL ANTIBODIES,
AUTOLOGOUS CELL PRODUCTS
Glioma-Stimulated Cytokine Production

- IL-2
- IFN-gamma
- IL-4
- IL-10
- IL-5
- TNF-alpha

Cytokine (pg/ml)

CD3, CD45RO, CD27, CD28

CXCR3, CXCR4, CCR2, CCR5
COHNMC PROTOCOL IRB#01020:  
(BB-IND #10109)

A PILOT FEASIBILITY/SAFETY STUDY OF CELLULAR IMMUNOTHERAPY FOR RECURRENT/REFRACTORY MALIGNANT GLIOMA USING GENETICALLY MODIFIED AUTOLOGOUS CD8+ T-CELL CLONES
<table>
<thead>
<tr>
<th>CYCLE:</th>
<th>WEEK:</th>
<th>DAY:</th>
<th>INTRACAVITARY CELL DOSE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10^7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>5 \times 10^7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10^8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>10^8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>10^8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10^8</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>10^8</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>10^8</td>
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<td>5</td>
<td>10^8</td>
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<tr>
<td>4</td>
<td>5</td>
<td>1</td>
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<td>3</td>
<td>10^8</td>
</tr>
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<td>5</td>
<td>10^8</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PROTOCOL #01020
CELL DOSING SCHEDULE

3 REST/RESTAGING

3 REST/RESTAGING
PLACEMENT OF RICKHAM SHUNT VAD IN RESECTION CAVITY
## PROTOCOL 01020 SAFETY DATA

<table>
<thead>
<tr>
<th>Cell Dose Level</th>
<th>Number of Doses</th>
<th>Observed Side-Effects Grade 3 or higher Attributable to Cell Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-cavitary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^7$</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>$5 \times 10^7$</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>$10^8$</td>
<td>19</td>
<td>Headache 2x – only on UPN 028</td>
</tr>
<tr>
<td><strong>Intra-parenchyma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$25 \times 10^6$</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>$50 \times 10^6$</td>
<td>1</td>
<td>WBC, Headache, Pain – localized to scalp/catheter site</td>
</tr>
<tr>
<td>$10^8$</td>
<td>3</td>
<td>Hypoxia, Headache (3x), Fatigue</td>
</tr>
</tbody>
</table>

### Cumulative Cell Dose

- UPN-028 - $9.6 \times 10^8$ cells over 21-days (Intra-cavitary)
- UPN-033 - $10.6 \times 10^8$ cells over 21-days (Intra-cavitary)
- $3.75 \times 10^8$ cells over 5-days (Intra-parenchymal)

### Best Response to therapy

- UPN-028 Radiographic CR  
  Survival Duration post Relapse: 12 months
- UPN-033 Radiographic CR  
  Survival Duration post Relapse: 11 Months
PROTOCOL 01020 UPN-033:
RADIOGRAPHIC COMPLETE RESPONSE FOLLOWING ADOPTIVE THERAPY

PRE-RX

14-WEEKS POST-RX

Bx. Proven Progressive GBM

5 T-CELL DOSES @ 10e8

Spectroscopy=Necrosis
FDG PET = Cold
INITIAL CLINICAL IMAGING EXPERIENCE USING $^{18}$F-FHBG PET ON COHNMC PROTOCOL #01020
PET-BASED MOLECULAR IMAGING OF HyTK EXPRESSING T-CELLS

HSV TK-  [\({}^{18}\text{F}\)] FHBG*  HSV TK+

P-FHBG*  P-FHBG*  P-FHBG*
UPN-033: Correlative Imaging of CNS Changes Observed Following Intracranial Adoptive Transfer Of Autologous IL13-zetakine+/HyTK+ CD8+ CTL Clone

Pre-Adoptive Rx
Ax Flair irFSE

Post-Adoptive Rx
Ax Flair irFSE

\(^{18}\text{FHBG PET}\)

Tumor Progression Versus T-Cell Mediated Inflammation

FHBG Signal From TK Reporter Expressed in T-Cells
UPN-033: Correlative Imaging of CNS Changes Observed Following Intracranial Adoptive Transfer Of Autologous IL13-zetakine+/HyTK+ CD8+ CTL Clone

Ax Flair irFSE

Tumor Progression Versus T-Cell Mediated Inflammation

\(^{18}\text{FHBG PET}

FHBG PET SIGNAL

CD8+ T CELL INFLTRATE
CHALLENGES TO BROADER CLINICAL UTILITY:

• PATIENTS FREQUENTLY NEED TO TAKE GLUCOCORTICOIDS (DEXAMETHASONE) TO MANAGE CEREBRAL EDEMA

• TIME TO PRODUCE AUTOLOGOUS PRODUCT NOT COMPATIBLE WITH RECURRENT DISEASE PROGRESSION KINETICS
Potential Advantages of Steroid-Resistant Alloclonoe:

- READY-TO-USE PRODUCT AVAILABLE AT TIME OF NEED

- FUNCTIONAL IN PATIENTS RECEIVING DEXAMETHASONE

- CONCURRENT USE OF DEXAMETHASONE LIMITS CNS INFLAMMATORY REACTION TO ADOPTIVE THERAPY/PROLONGS SURVIVAL OF T-CELL ALLOGRAFT BY INHIBITION OF REJECTION RESPONSE
INTRAPARENCHYMAL CNS LYMPHOID ALLOGRAFTS ARE NOT REJECTED
ZFP-Fok I Fusion Proteins

ZFN9666

ZFN9674
1. Endogenous gene targeted for disruption

2. ZFNs dimerize and introduce a double stranded DNA break in the gene

3. Break repaired by non-homologous end-joining (NHEJ) – resulting in loss of genetic information

4. Gene disrupted
Western Blot Shows Reduction of GR Protein Levels in GR-ZFN Treated CD8+ T-cells
RT-PCR Analysis Shows Loss of GR Target Gene Transcriptional Regulation in ZFN Modified T-Cells

**GILZ Expression**

- IL-13ZK Pool
- GFP 30
- ZFN 100
- 10A1

**IFNγ Expression**

- IL-13ZK Pool
- GFP 30
- ZFN 100
- 10A1
ZFN-MEDIATED GLUCOCORTICOID DISRUPTION OF ZETAKINE$^+$ CTLs RESULTS IN RESISTANCE TO DEXAMETHASONE-TRIGGERED APOPTOSIS
GR-KO CTLs RETAIN ZETAKINE-REGULATED LYTIC ACTIVITY

GR-ZFN Adeno Treated
MOI 100

GFP Adeno Control
MOI 100

Dex 10e-4M
GR- ZETAKINE+ CTL ARE DEX RESISTANT IN VIVO
GR-KO IL13-ZETAKINE
ALLOCLONE CLINICAL TRIAL

• UNRESECTABLE TUMORS
• DECADRON DEPENDENT PATIENTS
• INTRAPARENCHYMAL T-CELL INFUSIONS
• CONVECTION ENHANCED rHuIL-2 DELIVERY
Jensen Lab/Translational Research Team

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Stephen Forman, MD
Michael Kalos, PhD

Collaborators-
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Student
Tumor Tropism Project