Administration of Tumor-Specific Cytotoxic T Lymphocytes Engineered to Resist TGF-β to Patients with EBV-Associated Lymphomas

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Rationale of Immunotherapy for EBV-positive Lymphoma

- Significant failure rate of therapy for advanced stage or recurrent disease
- Long-term side effects of chemotherapy and radiation
- EBV antigens expressed by up to 40% of lymphomas are potential targets for T cell immunotherapy
EBV Specific Cytotoxic T Lymphocytes (CTL) Control EBV Infection in vivo

EBV Infected B cells

EBV Infected Lymphoma Cell

PBMC

TGFβ

CTL

EBV +ve Lymphoma Cell

LMP1

LMP2A

EBNA3

EBNA2

EBNA1

LMP1

LMP2

LP

Lytic

EBNA3

LMP2

LP

EBNA2

EBNA1

LMP2

LMP1

EBN2

EBNA1
LMP1 and LMP2A are potential CTL targets for Hodgkin and non-Hodgkin Lymphoma.

- LMP1 and LMP2A are potential CTL targets.

Hodgkin R-S Cell/NHL Cell
Making LMP1 and LMP2 Immuno-dominant Antigens

Ad5f35 LMP2

OR

Ad5f35 LMP1-I-LMP2

Dendritic Cells

PBMC

IL-2

LMP-specific Cytotoxic T Lymphocytes (CTL)

Lymphoblastoid cell line (LCL)

Bollard et al, JIT 2004
Gottschalk et al, Blood 2004 and Leen et al, JIT 2007
LMP1 & LMP2–Specific Activity in LMP-CTL from a Hodgkin Disease Patient

FLY-A2
LMP2 tetramer

CLG-A2
LMP2 tetramer

ILL-A29
LMP2 tetramer

YLL-A2
LMP1 tetramer

CD8

\[ 0.17\% \]

\[ 0.02\% \]

\[ 0.03\% \]  

\[ 0.01\% \]  

\[ 15.32\% \]

\[ 0.19\% \]

\[ 1.03\% \]

\[ 0.01\% \]

\[ 15.32\% \]

\[ 0.19\% \]

\[ 1.03\% \]

\[ 0.01\% \]

\[ 15.32\% \]

\[ 0.19\% \]

\[ 1.03\% \]

\[ 0.01\% \]

\[ 15.32\% \]

\[ 0.19\% \]

\[ 1.03\% \]

\[ 0.01\% \]
Relapsed Disease Arm (n=21)

- No toxicity
- 11 CR (1 also given Rituximab) (includes 1PR→CR)
- 2 very good partial responses (up to 36 mths)
- 8 progressive disease (2-8 wks)

Median clinical response: 1.5y (range: >6 to >40 mths)

Bollard et al, JCO 2013 in press
Clinical Responses post LMP-CTL in Patients with Active Disease

50% Disease Free Survival at 2 years

Proportion disease-free

0 1 2 3
0
0.2
0.4
0.6
0.8
1
P=0.744

LMP2-CTL study
LMP1/2-CTL study

n = 21
Immune Reconstitution of LMP1 and LMP2-specific T cells in Patients Treated with LMP1/2-CTL

Responders vs. Non-Responders

LMP1
SFC per 2x10^5

LMP2
SFC per 2x10^5
Evidence of Epitope spreading in Responding Patients Treated with LMP1/2-CTL

Responders

MAGE A4

Survivin

PRAME

Non-responders

MAGEA4

Survivin

PRAME

SFC per 2x10^5 cells

pre-infusion post-infusion
Can we make LMP-CTL resistant to the inhibitory effects of TGF-β secreted by Lymphoma cells?
Inhibits CTL proliferation

Inhibits cytotoxicity
- \( \downarrow \) perforin

Inhibits cytokine production
- \( \downarrow \) IFN \( \gamma \)
Creating a Mutant TGFβ Receptor II

Wild type Receptor

Truncated TGFβ Receptor II Dominant Negative Receptor (DNR)

Transmembrane domain

Stop codon 597

Retroviral vector SFG

SFG:DNR

MoMLV

U3 R U5

NcoI/BamHI

U3 R U5

ψ+

PBSQ

SD

SA

TM domain

DNR

Bollard et al, Blood 2002
Rendering LMP-specific T cells Resistant to TGFβ

Ad5f35 LMP1-I-LMP2

DC

EBV-LCL

SFG:DNR

PBMC

IL-2

DNR-transduced LMP CTLs

Study Eligibility

- EBV+ malignant cells EBER and/or LMP1 and/or LMP2 positive Lymphoma cells
- Patients with relapsed Hodgkin Disease or NHL including after allogeneic SCT
CD4 and CD8 T cells are DNR-transduced.
DNR-transduced CD4 and CD8 T cells are predominantly Effector Memory

n=6 CTL lines
DNR-Transduced CTL are LMP-specific

IFNγ release SFC per 1x10^5 cells

- **NT**
- **DNR-trans**

Bar chart showing IFNγ release SFC per 1x10^5 cells for various targets: CTL, LMP1, LMP2, hexon, EBV-LCL, PYL, TYG, GAL, LCL alone.
Patients Studied

• 5 females and 3 males
• EBV+ HL
  7 – relapsed post autologous SCT
  1 – relapsed post allogeneic SCT
• Two previously treated with LMP-CTL alone
• All refused additional chemotherapy
DNR-transduced T-cells Persist \textit{in vivo} for 5-23 months

**Patient 1**

**Patient 3**
Conclusions

• No dose limiting toxicity
• TGFβ-resistant LMP-CTL may beneficial in EBV+ Lymphoma
• DNR-trans LMP-CTL persist up to 3 years
• Now plan to explore the use of DNR-CTL in other TGFβ-secreting cancers