Targeting tumor antigen delivery to DC in vivo

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Are T-cells a “biomarker” for anti-lymphoma clinical responses?

• In 19 of 22 (86%) patients, Id-KLH + GM-CSF vaccination elicited CD8+ T-cells which lysed autologous lymphoma targets *Nature Med.* 1999; 5:1171.

• Most achieved molecular remissions; 3 did so without a detectable antibody response *Nature Med.* 1999; 5:1171.

• In 20 of 23 (87%) vaccinated patients, mantle cell lymphoma-specific T-cells producing type 1 cytokines were detectable by ICS *Nature Med.* 2005; 11:986
Lysis of Autologous Tumor Targets

Specific Lysis (%) vs. Effector:Target Ratio

Post Vaccine
UPN  Vaccine
6  8  15  16  19  23

Maximum level reached pre vaccine

Specific Lysis (%)
Effector:Target Ratio
Idiotype

Variable Region - Heavy Chain

NH₂ 1 31 35 50 65 95 102 S S 113

FW1 FW2 FW3 FW4

CDR1 CDR2 CDR3
T-cell lines specifically recognize autologous Id protein
LE-I.1 T cells Produce Th1 Cytokines in Response to Specific CDR2 Peptide
Immunodominant T-cell epitopes localize to CDR, not FWR, regions of $V_{H}$
### T Cell Epitopes in Lymphoma Ig V<sub>H</sub> Regions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Synthetic Peptide</th>
<th>Position</th>
<th>Response</th>
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<tbody>
<tr>
<td>TL</td>
<td>ATTTGGGLNFGGLDVW</td>
<td>CDR3</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>FLQMNSLRV</td>
<td>FWR3</td>
<td>NO</td>
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<tr>
<td></td>
<td>VPGKGLVWV</td>
<td>FWR2</td>
<td>NO</td>
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<tr>
<td>BL</td>
<td>GDDWSGYFK</td>
<td>CDR3</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>YVDSVKGRF</td>
<td>CDR2</td>
<td>YES</td>
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<tr>
<td></td>
<td>SQSGSDTSY</td>
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<td>NO</td>
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<tr>
<td></td>
<td>LRVEDTAIY</td>
<td>FWR3</td>
<td>NO</td>
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<tr>
<td></td>
<td>GSDTSYVDS</td>
<td>CDR2</td>
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<tr>
<td>BS</td>
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<td>LQMSSLRVEDTALYY</td>
<td>FWR3</td>
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<tr>
<td></td>
<td>RFTISRDNKINIVFL</td>
<td>FWR3</td>
<td>NO</td>
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Baskar et al. J Clin Invest. 2004
Harnessing DC for Tumor Vaccine Therapy

In vitro

- Tumor Cell
- DC
- Protein/Peptide Virus
- cDNA
- mRNA

In vivo

- Immature DC
- Mature DC
- NKT
- NK
- Mφ
- CD8+ T-Cell
- CD4+ T-Cell
- MHC-I
- MHC-II
- Receptor Mediated Internalization
- Cell Surface Receptor
- Mileau of Proinflammatory Cytokines and Chemokines with Recruitment of Other Cells
- Migration to Lymphoid Organs and Maturation
- Proinflammatory Cytokines and Factors

38C-13 lymphoma-derived:

- sFv38
- IP10sFv38
- IP10sFv38(INV)
- IP10dsFv38
- IP10TsFv38
- MCP3sFv38
- MCP3sFv38(INV)
- PreS2sFv38
- DomAsFv38
- psFv38(INV)
- pMCP3sFv38(INV)
- pPreS2sFv38(INV)

A20 lymphoma-derived:

- IP10sFv20
- MCP3sFv20
- psFv20A
- pMCP3sFv20

Vaccine delivery:

- protein
- protein
- protein
- protein
- protein
- protein
- protein
- protein
- Naked DNA
- Naked DNA
- Naked DNA
IP-10 and MCP-3–fused scFv bind specifically to murine chemokine receptors
In vivo Chemotaxis by scFV Fusion Proteins (72h, Injected 10μg s.c.)

PreS2-scFV38 (x100)  
MCP3-scFV (x100)  
IP10-scFV38  
IP10-scFV38 (x400)
Injection of plasmid DNA encoding iDC chemo-attractant fusions elicit therapeutic antitumor immunity

Survival %

Day

p<0.002

p<0.0003

Injection of plasmid DNA encoding iDC chemo-attractant fusions elicit therapeutic antitumor immunity
Protective immunity induced by naked DNA MCP3-sFv38 vaccine requires effector CD8$^+$ T cells.
Murine defensin induces maturation of bone marrow-derived immature DC

- medium alone: 15%
- mDefensin2β: 68%
- mproDefensin2β: 18%

Biragyn et al. Science 2002
Possible Modes of Action of Chemokine-Ag Fusions In Vivo

APA Receptor Targeting

APA Maturation/Activation

Chemotaxis of APA and Other Cells
Summary of Published Results: Second-Generation Genetic Vaccines

- Genetic fusion of weak tumor antigens to chemokines or related chemoattractant peptides (eg, defensins) converts them into potent vaccines.

- CD8+ effector T-cell immunity is required.

- Defensins induce DC maturation through TLR-4, providing a link between innate and adaptive immunity.

- Mixing separate plasmids encoding antigen and chemokine failed to elicit immunity.
Future Plans

• Determine the mechanism(s) of action of sFv-chemokine fusion DNA vaccines (vivo and vitro)

• Test selected additional chemokine receptor ligands for fusion to sFv antigen

• Design a pilot clinical trial of sFv-chemokine fusion DNA vaccination
Priming in vivo to melanoma Ag gp100 is facilitated by chemokine fusion

<table>
<thead>
<tr>
<th>DNA Vaccine (i.m. x 3)</th>
<th>CD8 peptide (gp100 25-33)</th>
<th>Medium</th>
<th>Mitogen</th>
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<tr>
<td>MIP3α(h)gp100</td>
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<tr>
<td>(M)MIP3α(h)gp100</td>
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<td>Untreated</td>
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![Image of spots](image.png)

![Graph of spots](graph.png)

Number of spots / 2x10E5 cells
Cross-presentation of chemokine – gp100 fusions

Schiavo et al.
Blood 2006
Translational Development of Vaccines for Lymphoma and Myeloma

LAB
2nd Generation Vaccines
- Id-Chemokine Fusions
- HIV DNA Vaccines

T-Cell Adjuvants
- GM-CSF

Characterization of Id T-cell epitopes

Novel Antigen Discovery

• SCT Donor Vaccination

CLINIC
- FL in 1st Remission (Phase III)
- MCL, DLCL (Phase I)

Donor Vaccination (Phase I myeloma)
The Center for Cancer Immunology Research

Yong Jun Liu, Director

Associate Directors:
Jeffrey Molldrem
Larry Kwak
Patrick Hwu
Steven Ullrich
<table>
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<tr>
<th>Fellow</th>
<th>Current institution</th>
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<tr>
<td>Hong Qin, PhD</td>
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<td>Soung-Chul Cha, PhD</td>
<td>Univ. Navarra (Spain)</td>
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<td>Arya Biragyn, PhD</td>
<td>Univ. Milan (Italy)</td>
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<td>M. Bendandi, MD</td>
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<td>P. Ruffini, MD</td>
<td>U.S. Army medical research</td>
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<td>L. Sternas, MD, PhD</td>
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<td>S. Weeks, PhD</td>
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<td>J. Kim, PhD</td>
<td>Johns Hopkins Univ.</td>
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<td>H. Goto, MD</td>
<td>NCI research contractor</td>
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<td>C. Kobrin, PhD</td>
<td>Industry</td>
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<td>R. Hornung, PhD</td>
<td>Yonsei University</td>
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<td>M. Dar, M.D.</td>
<td>Dongguk University</td>
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<td>Seung-Tae Lee, MD</td>
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<td>Keon Uk Park, MD</td>
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</table>
“Plans fail for lack of counsel, but with many advisors they succeed”

*Proverbs 15:22*

Ronald Levy, MD  
Dan Longo, MD  
Edison Liu, MD  
Richard Klausner, MD