BIOTHERAPY:
4TH MODALITY OF CANCER TREATMENT

Therapeutic Approaches with Immune Cells

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Adoptive Cellular Therapy

- The administration of cells with antitumor activity as anticancer therapy
- Generally involves ex vivo manipulation to enhance activity or enrich cell type
- Most clinical experience has been with lymphocytes
Clinically tested forms of adoptive cellular therapy

- Lymphokine activated killer cells [LAK]
- Tumor infiltrating lymphocytes [TIL]
- Autolymphocyte Therapy [ALT]
- Dendritic cell therapy [DC]
- Non-myeloablative allogeneic stem cell transplant and donor lymphocyte therapy [DLT]
How do lymphocytes kill malignant cells?

• **Granule Exocytosis:** transfer of **perforin** (cytolysin = pore-forming protein-PFP) in cytoplasmic granules → transfer of **serine proteases** (gzms) → membrane leaks & apoptosis [NK, LAK, CD8+CTL]

• **Fas Pathway:** binding to Fas (a surface antigen related to TNF receptor) → apoptosis [CD4+ & CD8 CTL]
Immune Cells for Adoptive Cellular Therapy
Lymphokine Activated Killer Cells [LAK]

- A subset of Natural Killer [NK] cells with nonspecific cytotoxicity against tumor cells, whose activity is enhanced by Interleukin-2
- Defined in vitro by the difference in cytotoxicity for PBL vs IL2-stimulated PBL against K562 (NK-sensitive) and Daudi (NK-resistant) cell lines
- CD56+, CD25+, CD20-, CD16+, CD3+
<table>
<thead>
<tr>
<th>DX</th>
<th># Pts</th>
<th>CR #</th>
<th>PR #</th>
<th>RR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>72</td>
<td>8</td>
<td>17</td>
<td>35%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>48</td>
<td>4</td>
<td>6</td>
<td>21%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>30</td>
<td>1</td>
<td>4</td>
<td>17%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>57%</td>
</tr>
<tr>
<td>Others</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>178</strong></td>
<td><strong>14</strong></td>
<td><strong>30</strong></td>
<td><strong>25%</strong></td>
</tr>
</tbody>
</table>

Rosenberg SA et al. High Dose Bolus IL-2
# CBRG: IL-2 + LAK

<table>
<thead>
<tr>
<th>DX</th>
<th># Pts</th>
<th>CR #</th>
<th>PR #</th>
<th>RR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>46</td>
<td>2</td>
<td>5</td>
<td>15%</td>
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<tr>
<td>Melanoma</td>
<td>54</td>
<td>3</td>
<td>10</td>
<td>24%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>9</td>
<td>0</td>
<td>2</td>
<td>22%</td>
</tr>
<tr>
<td>Other</td>
<td>98</td>
<td>2</td>
<td>8</td>
<td>10%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>242</strong></td>
<td><strong>7</strong></td>
<td><strong>24</strong></td>
<td><strong>13%</strong></td>
</tr>
</tbody>
</table>

Dillman RO et al. Continuous Infusion IL-2
Pulsed LAK

- 15-60 min incubation ex vivo in pheresis bag with IL2 6000 IU/ml
- enhanced cytotoxicity only if preceded by in vivo infusional IL2 to prime LAK cells
- tremendous cost savings compared to 2-4 day LAK incubation in vitro

Horton et al: Cancer Res 1990
Pulsed LAK: clinical trials

- Oldham et al: Proc ASCO 1991. Primed with IL2 18 MIU/m^2/day x 5 days → 10 liter leukapheresis → 6000 IU/ml pulse ex vivo → infuse w/ CIV IL2. 5/63 responses

- Yeung et al: Cancer 1993. Primed with CIV IL2 6 MIU/m^2/day x 4 days → 10-liter leukapheresis → 9 MIU/ml pulse ex vivo IL2 → infuse w/ CIV IL2. 6/19 responses
## Randomized Trials of IL-2 ± LAK Cell Therapy

<table>
<thead>
<tr>
<th>1st Author</th>
<th>Year</th>
<th># of Patients</th>
<th>Treatments*</th>
<th>IL-2/mo in MIU</th>
<th>Result/Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosenberg</td>
<td>1993</td>
<td>85/91</td>
<td>HDB IL-2 + LAK</td>
<td>1836</td>
<td>RCC: 33% vs 24% RR; OS NSD [p=.52]</td>
</tr>
<tr>
<td>Renal Cell &amp; Melanoma</td>
<td>NCI</td>
<td>79/90</td>
<td>HDB IL-2</td>
<td>1836</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDCIV IL-2 + LAK</td>
<td>120</td>
<td>3% vs 9% RR [p=.61]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDCIV IL-2</td>
<td>120</td>
<td>13 vs 11 mos med OS [p=0.67] LAK more toxic</td>
</tr>
<tr>
<td>Law</td>
<td>1995</td>
<td>35</td>
<td>LDCIV IL-2 + LAK</td>
<td>24</td>
<td>No responses in either arm</td>
</tr>
<tr>
<td>Renal Cell</td>
<td>MSK</td>
<td>36</td>
<td>LDCIV IL-2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Koretz</td>
<td>1991</td>
<td>19</td>
<td>LDCIV IL-2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Renal Cell &amp; Melanoma</td>
<td>Emory</td>
<td>19</td>
<td>LDCIV IL-2</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
NCI: IL-2 ± LAK

- IL-2
- IL-2 + LAK

\[ p2 = 0.062 \]

**Melanoma**  N = 54
Rosenberg JNCI 1993

**Renal Cell**  N = 87
Rosenberg JNCI 1993
# Published Experience with Brain LAK in Recurrent GBM

<table>
<thead>
<tr>
<th>Citation</th>
<th>Method of LAK</th>
<th># pts</th>
<th>60 day mortality</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barba 1989</td>
<td>J Neurosurg Stereotactic + IL-2</td>
<td>9</td>
<td>33%</td>
<td>&lt;4 mos</td>
</tr>
<tr>
<td>Lillehei 1991</td>
<td>Neurosurg Plasma clot → IL-2/LAK via catheter</td>
<td>20</td>
<td>--</td>
<td>&lt; 5 mos</td>
</tr>
<tr>
<td>Merchant 1988</td>
<td>Cancer Surgical + IL-2</td>
<td>13</td>
<td>16%</td>
<td>&lt; 6 mos</td>
</tr>
<tr>
<td>Jeffes 1993</td>
<td>J Neuro-Oncol Surgical + IL-2</td>
<td>19</td>
<td>--</td>
<td>7.5 mos</td>
</tr>
<tr>
<td>Dillman</td>
<td>J Immunother Surgical ± IL-2</td>
<td>40</td>
<td>2%</td>
<td>9.0 mos</td>
</tr>
<tr>
<td>Hayes 1995</td>
<td>Cancer + IL-2 via Ommaya</td>
<td>15</td>
<td>--</td>
<td>12.2 mos</td>
</tr>
</tbody>
</table>
## Survival After Resection of Recurrent GBM

<table>
<thead>
<tr>
<th>Study</th>
<th>Journal</th>
<th>Year</th>
<th>Patients</th>
<th>Median Survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sipos</td>
<td>Neurochir</td>
<td>1997</td>
<td>60 pts</td>
<td>4.3 mos</td>
</tr>
<tr>
<td>Dirks</td>
<td>Can J Surg</td>
<td>1993</td>
<td>43 pts</td>
<td>4.4 mos</td>
</tr>
<tr>
<td>Brem</td>
<td>Lancet</td>
<td>1995</td>
<td>112 pts</td>
<td>5.3 mos</td>
</tr>
<tr>
<td>Brem</td>
<td>Lancet</td>
<td>1995</td>
<td>110 pts*</td>
<td>7.2 mos</td>
</tr>
<tr>
<td>Ammirati</td>
<td>Neurosurg</td>
<td>1987</td>
<td>35 pts</td>
<td>7.2 mos</td>
</tr>
<tr>
<td>Harsh</td>
<td>Neurosurg</td>
<td>1987</td>
<td>39 pts</td>
<td>8.4 mos</td>
</tr>
<tr>
<td>Salc</td>
<td>Neurosurg</td>
<td>1994</td>
<td>40 pts</td>
<td>8.6 mos</td>
</tr>
<tr>
<td>Dillman</td>
<td>J Immunother</td>
<td>2004</td>
<td>40 pts</td>
<td>9.0 mos</td>
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</table>
Cytotoxic T-Lymphocytes

- CD8+ T-Lymphocytes
- Cytotoxicity restricted to recognition of foreign or tumor antigen in combination with HLA Class I histocompatibility antigens of self
- Highly specific, self-restricted activity
- 10- to 100-fold more cytotoxic than LAK
Tumor Infiltrating Lymphocytes [TIL]

• Presumption that some lymphocytes which infiltrate tumor masses \textit{in vivo} are antigen-specific, HLA-restricted CTL

• Cultures of single cell suspensions of fresh tumors in IL-2 results in selection of T-lymphocytes with varying degrees of tumor-specific and patient-specific cytotoxicity

• May be predominantly CD8+ or CD4+, may be cytotoxic or non-cytotoxic
Hollow Fiber Bioreactor to Grow TIL
## Success rates in growing TIL

<table>
<thead>
<tr>
<th>First Author</th>
<th>Site</th>
<th>years</th>
<th># TIL</th>
<th>% TIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewko</td>
<td>CTI</td>
<td>91-94</td>
<td>90/113</td>
<td>80%</td>
</tr>
<tr>
<td>Malone</td>
<td>Hoag</td>
<td>96-99</td>
<td>26/34</td>
<td>76%</td>
</tr>
<tr>
<td>Dillman</td>
<td>BTX</td>
<td>87-90</td>
<td>56/82</td>
<td>72%</td>
</tr>
<tr>
<td>Schiltz</td>
<td>Hoag</td>
<td>91-95</td>
<td>67/94</td>
<td>71%</td>
</tr>
<tr>
<td>Oldham</td>
<td>BTX</td>
<td>87-90</td>
<td>129/196</td>
<td>66%</td>
</tr>
<tr>
<td>Yanelli</td>
<td>NCI</td>
<td>89-93</td>
<td>160/255</td>
<td>63%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td>502/740</td>
<td>68%</td>
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</table>
Localization of Indium-111 Labeled TIL

• Fisher B et al (J Clin Oncol 1989)
  – tumor sites imaged in 6/6 melanoma patients within 24-144 hrs
  – initial localization to lung, liver, and spleen during first two hours

• Pockam BA et al (Cancer 1994)
  – tumor uptake in 26/38 melanoma patients
  – more likely to localize if CTX pre TIL: 20/26 (81%) vs 5/12 (42%) (p=.026)
  – those who imaged received more TIL (p=.005)
  – response 10/26 who imaged vs 0/12 who did not (p=.022)

• Dillman et al (Cancer Biother Radiopharm 1997)
  – Tumor uptake in 8/8 patients (5 RCC, 2 melanoma, 1 colon)
  – Sites imaged included: bone, brain, lung, liver, lymph nodes and soft tissue mass
  – No objective responses
# TIL Therapy: phase II trials

<table>
<thead>
<tr>
<th>Auth</th>
<th>Org</th>
<th>Dx</th>
<th>IL2</th>
<th>Rx</th>
<th>#Pts</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosenberg</td>
<td>NCI</td>
<td>Mel</td>
<td>HB+CTX</td>
<td></td>
<td>86</td>
<td>34%</td>
</tr>
<tr>
<td>Pierce</td>
<td>UCLA</td>
<td>RCC</td>
<td>LCIV+IFN</td>
<td></td>
<td>48</td>
<td>33%</td>
</tr>
<tr>
<td>Dillman</td>
<td>CBRG</td>
<td>Mel</td>
<td>HCIV+CTX</td>
<td></td>
<td>21</td>
<td>24%</td>
</tr>
<tr>
<td>Kradin</td>
<td>MGH</td>
<td>RCC, MEL</td>
<td>LCIV</td>
<td></td>
<td>28</td>
<td>21%</td>
</tr>
<tr>
<td>Goedegeburre</td>
<td>Brigham</td>
<td>Mel, RCC</td>
<td>LB</td>
<td></td>
<td>26</td>
<td>11%</td>
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<td>Oldham</td>
<td>CBRG</td>
<td>Not Mel</td>
<td>HCIV+CTX</td>
<td></td>
<td>30</td>
<td>3%</td>
</tr>
<tr>
<td>Bukowski</td>
<td>Cleve</td>
<td>RCC</td>
<td>CIV</td>
<td></td>
<td>18</td>
<td>0%</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>257</td>
<td>24%</td>
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## Randomized Trials of TIL Cell Therapy

<table>
<thead>
<tr>
<th>1st Author</th>
<th>Year</th>
<th># of Patients</th>
<th>Treatments*</th>
<th>IL-2/mo in MIU</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figlin Metastatic</td>
<td>1999</td>
<td>39/72/81</td>
<td>MDCIV IL-2 + TIL</td>
<td>160</td>
<td>NSD RR or OS by intent-to-treat analysis, but 41% did not receive intended TIL</td>
</tr>
<tr>
<td>Renal Cell consortium</td>
<td></td>
<td>68/79</td>
<td>MDCIV IL-2 + placebo</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Ratto Stage II-III</td>
<td>1996</td>
<td>113/131</td>
<td>SC IL-2 + TIL -&gt; VBL/CSP + RT</td>
<td>2-16 MIU/m²</td>
<td>22.4 vs 14.1 mos OS p.05</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Italy</td>
<td>113/113</td>
<td>VBL/CSP + RT</td>
<td>qd x 14 as tolerated</td>
<td></td>
</tr>
<tr>
<td>Dreno Stage III</td>
<td>2002</td>
<td>44</td>
<td>SC IL-2 + TIL</td>
<td>IL-2 x 2 mos</td>
<td>NSD median F/U 4 yrs ↓ relapse if only 1 + node</td>
</tr>
<tr>
<td>Melanoma</td>
<td>France</td>
<td>44</td>
<td>SC IL-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Correlates of TIL antitumor effects in vivo

- number of cells: \( > 10^{11} \) cells
- cytotoxicity against allogeneic or autologous targets
- ease and rapidity of cell expansion
- not clear that HLA-restricted TIL [CTL] any better than nonspecific TIL [T-LAK]
Autolymphocyte Therapy

• Non-cytotoxic T cells, of memory-helper phenotype, derived from peripheral blood mononuclear cells by cell culture in the presence of autologous lymphokines (ALK) secreted by autologous mononuclear cells after they have been stimulated with anti-CD3 monoclonal antibody in the presence of putative inhibitors of suppressor T cells; there is no incubation with IL-2
AutoLymphocyte Therapy

- Autolymphokine (ALK) Media: About $5 \times 10^9$ autologous lymphocytes stimulated \textit{in vitro} for 3 days with anti-CD3 monoclonal antibody in the presence of indomethicin and cis-retinoic acid to obtain media containing significant amounts of TNF-\(\alpha\), IL-1\(\beta\), interferon-\(\gamma\), and IL-6, but no IL-2

- ALT: for 6 months, 3-5 \times 10^9 PBMC incubated with aliquot of supplemented ALK media for 6 days → CD3+, CD4+, CD29+ (4B4) “helper” lymphocytes, not cytotoxic

- Infused i.v. monthly while patient on cimetidine 600 mg po q6h

Auto-Lymphocyte Therapy

- 90 patient randomized trial in metastatic kidney cancer
- Cimetidine + ALT vs Cimetidine
- 21% vs 5% OR
- Better OS p=.008

Osband ME et al, Lancet 335:994, 1990
Auto-Lymphocyte Therapy

• Lavin PT et al. Transplant Proc 24:3059-3064, 1992
  – 335 patients, 3 sites, experience in metastatic renal cell carcinoma
  – 10/259 (4%) response rate

  – 47 patients, metastatic solid tumors, response rate of 4%
  – 1/13 renal cell
  – 0/13 colon
  – 0/6 breast
  – 0/5 lung
Auto-Lymphocyte Therapy

• Randomized trial vs observation in node + or T4 regionally advanced disease stopped with 20 patients in each arm

• 180 patient randomized trial vs interferon-alpha in metastatic disease—NSD
Dendritic Cells

- High levels of accessory molecules: iCAM1, LFA1
- High levels of costimulatory molecules: B7a, CD80, CD86
- Low FcγRs and CSFs, weak phagocytosis, nonadherent
- GM-CSF responsive: viability, function, growth
Dendritic Cells

- Derived from peripheral blood mononuclear cells or stem cells by incubation with GM-CSF, IL-4, ± TNF-α
- Usually applied in vaccine strategy
- Some trials involving iv infusion
Intravenous Dendritic Cells

- DC loaded with Prostate membrane specific antigen, responses alleged in 5 patients in phase I trial—Tjoa, Prostate 1997
- In 5 patients with melanoma, i.v. infusion of radiolabeled DC resulted in transient localization in lung followed by retention in liver, and spleen while intralymphatic injected resulted in uptake and retention in lymph nodes for more than 24 hrs—Mackensen, Cancer Immunol Immunother 1999
- DC loaded with melanoma peptides, one CR in 16 patients, infusion well-tolerated—Lau R, J Immunother 2001
- Colon and Pancreas cancer: 6 pts received DC from CD34+ cells; 6 pts received DC from PBMC—Triozzi J Hematoother Stem Cell Res 2003
Donor Lymphocyte Infusions or Mini-Allogeneic Transplants

- Allogeneic transplant but using marrow suppressive rather than ablative doses
- HLA matched donor
- Immunosuppression to decrease GVHD
- Relying on Graft vs Tumor Effect
- Can give infusions of donor lymphocytes (DLI) to supplement anti-tumor effect or to eliminate/reduce chimerism
Hematopoietic Stem Cells
Allogeneic Lymphocytes

• 19 patients, refractory metastatic renal-cell cancer
  – HLA-identical sibling or a sibling with a mismatch of a single HLA antigen suitable donors

• Regimen
  – FluCy to temporarily ablate hematopoiesis and lymphocytes
  – IV infusion of allogeneic PBSC
  – Cyclosporine to decrease GVHD, withdrawn early in patients with mixed T-cell chimerism or PD
  – Patients with no response received up to three infusions of donor lymphocytes

• Results
  – 3 CR, 10 PR, 2 transplant related deaths
  – Regression of lesions was delayed (median 4 mos) suggesting benefit from immune therapy rather than chemotherapy

Allogeneic Lymphocytes

• 20 patients, with B-cell lymphoma, recurred after autologous transplant, but still responsive to chemotherapy
  – HLA-identical sibling or a sibling with a mismatch of a single HLA antigen suitable donors

• Regimen
  – FluCy + rituximab, FluCy+AraC to temporarily ablate host hematopoiesis and lymphocytes
  – IV infusion of allogeneic PBSC
  – Tacrolimus and methotrexate to decrease GVHD, adjusted or stopped if mixed T-cell chimerism or PD
  – Patients with no response received up to three infusions of donor lymphocytes

• Results
  – 95% PFS at median F/U of 2 years

Allogeneic Lymphocytes

- 53 pts post stem cell transplants from HLA-matched related or unrelated donors
  - DX: 10 MDS, 10 AML/ALL, 11 CLL, 9 myeloma 9 lymphoma, 4 solid tumors

- Regimen
  - 2 Gy TBI ± fludarabine to temporarily ablate host hematopoiesis and lymphocytes
  - Mycophenolate mofetil and cyclosporine to decrease GVHD
  - Donor lymphocyte infusion (DLI) with a median CD3 dose of 10 million cells/kg, for persistent disease (n = 8), disease relapse (n = 17), progressive disease (n = 12), low donor chimerism with disease (n = 11), or low chimerism with remission (n = 5)

- Results
  - 25% (7/48) response rate, 32% OS at median 30 mos F/U
  - 17% grade II-IV GVHD

Strategies to improve DLI or Mini Allo Transplants

• T cell reduction to reduce GVHD
• Variations in preparative regimens: monoclonal antibodies, chemotherapy, radiation therapy, radioimmunotherapy
• Variations in immunosuppressive agents
• Subset selection of donor lymphocytes
Obstacles to Adoptive Cellular Therapy

- Need for cell biology support system
- Autologous cells: cost and inefficiency—must make a new product for each individual patient
- Allogeneic cells: compatibility matching and morbidity of GVHD
Conclusions

• Adoptive cellular therapy with autologous lymphocytes has been associated with significant tumor responses in clinical trials, but there is no FDA approved product (process)

• Donor Lymphocyte Infusions have had limited benefit in treatment of refractory solid tumors, but activity in hematopoietic malignancies

• Cost:Benefit of technology remains a formidable obstacle to use and investigation
Hoag Cancer Center
Newport Beach, CA