T CELL THERAPY FOR THE CONTROL OF EBV-RELATED NASOPHARYNGEAL CARCINOMA

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EBV-associated cancers

- Burkitt lymphoma (1964)
- Nasopharyngeal carcinoma (1970)
- Lymphoproliferative diseases in hosts with impaired T-cell immunity (1982 →)
- T-cell Lymphoma (1988)
Cellular immunotherapy - EBV-related LD

- Treatment with EBV-specific CTL induced regression of relapsed EBV positive Hodgkin disease (Roskrow et al., Blood 1998)
- Infusion of EBV-specific CTL prevented development of EBV-related post-transplant lymphoproliferative diseases (Comoli et al., Blood 2002)
Cellular therapy for EBV-related NPC: RATIONALE

- NPC tumor cells express a restricted number of viral proteins, namely EBNA1, LMP1 and LMP2 - Cohen: N Engl J Med 2000
  - NPC cells show high levels of HLA class I alleles on the cell surface and have normal expression of the MHC-encoded putative peptide transporters TAP-1 and TAP-2, as well as of other components of the class I processing pathway - Khanna, Cancer Res 1998
  - EBV-specific CTLs are present in patients with newly diagnosed NPC, with a specificity for EBV latent protein LMP2 - Lee, J Immunol 2000

NPC cells are capable of immunological processing and CTL recognition
Generation of EBV polyspecific CTLs (Rooney 1995)

1st step
4-10 weeks
PBMC + EBV → Immortalized B-cell: EBV-LCL

2nd step
4-6 weeks
PBMC + IL-2 → CTL expansion → Cryopreserve → Infuse

Test for:
- specificity
- phenotype
- sterility
- identity

QA/QC validated

<table>
<thead>
<tr>
<th>CTL phenotype</th>
<th>%</th>
<th>CD3+CD8+</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+</td>
<td>90 (68-99)</td>
<td>CCR7+CD45RA+</td>
<td>4±4</td>
</tr>
<tr>
<td>CD4+</td>
<td>30 (4-90)</td>
<td>CCR7+CD45RA-</td>
<td>7±7</td>
</tr>
<tr>
<td>CD8+</td>
<td>60 (8-96)</td>
<td>CCR7-CD45RA-</td>
<td>79±18</td>
</tr>
<tr>
<td>CD8+/56+</td>
<td>10 (0-33)</td>
<td>CCR7-CD45RA+</td>
<td>10±7</td>
</tr>
</tbody>
</table>

PBMC + EBV

Immortalized B-cell: EBV-LCL

CTL expansion

Cryopreserve

Infuse

QA/QC validated

Test for:
- specificity
- phenotype
- sterility
- identity

PBMC + IL-2

Mismatched B-cells

CD8+/56+CCR7-CD45RA-

CD8+/56+CCR7+CD45RA-

CD8+/56+CCR7-CD45RA+

CD8+/56+CCR7+CD45RA+

EBNA 3A (LLD) (FLR) (SVR)

LMP2 (protein)

PBMC

Immortalized B-cell: EBV-LCL

CTL expansion

Cryopreserve

Infuse

QA/QC validated

Test for:
- specificity
- phenotype
- sterility
- identity

PBMC + IL-2

Mismatched B-cells

CD8+/56+CCR7-CD45RA-

CD8+/56+CCR7-CD45RA+

CD8+/56+CCR7+CD45RA-

CD8+/56+CCR7+CD45RA+

EBNA 3A (LLD) (FLR) (SVR)

LMP2 (protein)
Cell Therapy of Stage IV Nasopharyngeal Carcinoma With Autologous Epstein-Barr Virus–Targeted Cytotoxic T Lymphocytes

Patrizia Comoli, Paolo Pedrazzoli, Rita Maccario, Sabrina Basso, Ornella Carminati, Massimo Labirio, Roberta Schiavo, Simona Secondino, Chiara Frasson, Cesare Perotti, Mauro Moroni, Franco Locatelli, and Salvatore Siena
Therapy with EBV-specific CTLs in NPC: OUR PREVIOUS EXPERIENCE

- 10 patients with refractory and poor prognosis NPC
- QW or Q2W infusions of “low-dose” poly-specific CTLs ($20-80 \times 10^6$)
  - are feasible and safe
  - provide clinical benefit in some patients

P Comoli et al. J Clin Oncol 2005
Therapy with EBV-specific CTLs in NPC

HUSTON EXPERIENCE

- 10 patients treated with poly-specific CTLs (4 receiving CTLs in remission)

- Clinical results:
  - decrease of viral load
  - 2 documented CR, 1 PR, 1 SD

Straathof et al. Blood 2005
PRESENT STUDY

- infusion of higher CTL doses
- following lymphodepleting chemotherapy
  - Yee, PNAS 2002
  - Rosenberg, PNAS 2004
  - Dudley, JCO 2005
PATIENTS – INCLUSION CRITERIA

- Less than 70 years with histologically-confirmed EBV-related NPC
- Disease in progression after two lines of chemotherapy and not amenable to complete surgical resection or local conventional treatments
- Measurable disease (RECIST criteria)
- Normal organ function
- Informed consent
LYMPHODEPLETING CHEMOTHERAPY
- Cyclophosphamide 60 mg/Kg
- Fludarabina 120 mg/mq

Days
1 4

CTL 1st dose
L<0.1
mm³

CTL 2nd dose

Disease evaluation

IL-2:10^6 U sc die

Maintenance “low-dose” CTLs in pts with response
### Main characteristics of treated patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Stage at diagnosis</th>
<th>Site(s) of tumor involvement at the time of cell therapy</th>
<th>Prior therapies</th>
<th>ECOG PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.RZ</td>
<td>19</td>
<td>F</td>
<td>IV (T4N2M0)</td>
<td>Liver, spleen</td>
<td>RT 3 lines of CT</td>
<td>0</td>
</tr>
<tr>
<td>2.AM</td>
<td>65</td>
<td>M</td>
<td>III (T3N1M0)</td>
<td>Primary tumor, skull base</td>
<td>2 lines of CT, RT, surgery</td>
<td>0</td>
</tr>
<tr>
<td>3.JW</td>
<td>21</td>
<td>M</td>
<td>III (T3N1M0)</td>
<td>Primary tumor, skull base</td>
<td>2 lines of CT, RT</td>
<td>0</td>
</tr>
<tr>
<td>4.ST</td>
<td>40</td>
<td>F</td>
<td>III (T2N2M0)</td>
<td>Skull base, neck</td>
<td>3 lines of CT, RT</td>
<td>1</td>
</tr>
<tr>
<td>5.GG</td>
<td>48</td>
<td>M</td>
<td>IV (T2N2M1)</td>
<td>Primary tumor, skull base</td>
<td>2 lines of CT, RT, surgery</td>
<td>1</td>
</tr>
<tr>
<td>6.PC</td>
<td>64</td>
<td>M</td>
<td>III (T3N0M0)</td>
<td>Primary tumor</td>
<td>2 lines of CT, RT</td>
<td>0</td>
</tr>
<tr>
<td>7.VL</td>
<td>49</td>
<td>M</td>
<td>Unknown</td>
<td>Skull base, lung, lymph nodes, orbital cavity</td>
<td>3 lines of CT, RT surgery</td>
<td>1</td>
</tr>
<tr>
<td>8.MC</td>
<td>40</td>
<td>M</td>
<td>Unknown</td>
<td>Primary tumor, skull base</td>
<td>3 lines of CT, RT</td>
<td>0</td>
</tr>
<tr>
<td>9.GFMB</td>
<td>66</td>
<td>M</td>
<td>IV (TXN2M1)</td>
<td>Primary tumor, lymph nodes</td>
<td>3 lines of CT</td>
<td>0</td>
</tr>
<tr>
<td>10.MM</td>
<td>46</td>
<td>M</td>
<td>II (T2N1M0)</td>
<td>Lung, lymph nodes, liver</td>
<td>2 lines of CT, RT, surgery</td>
<td>1</td>
</tr>
</tbody>
</table>
Characteristics of CTL lines

<table>
<thead>
<tr>
<th></th>
<th>CD8</th>
<th>HLA-DR</th>
<th>CD4</th>
<th>CD8/CD56</th>
<th>CD56</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV-LCL</td>
<td>70%</td>
<td>98%</td>
<td>16%</td>
<td>8%</td>
<td>14%</td>
</tr>
<tr>
<td>EBV-LMP2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E/T ratio 5:1
RESULTS (1)

- Median time to CTL production: 3.5 months
- Chemotherapy well tolerated
  - No grade III-IV non-hematological toxicity
  - Grade IV uncomplicated neutropenia in 3
  - Manageable in the outpatient setting
- Dose of CTL per infusion:
  - median $370 \times 10^6$
  - range: $160-500 \times 10^6$
## RESULTS (2): CTL therapy and Outcome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total CTL dose</th>
<th>Adverse Events</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.RZ</td>
<td>$5.6 \times 10^8$</td>
<td>None</td>
<td>SD (4 months)</td>
</tr>
<tr>
<td>2.AM</td>
<td>$13.8 \times 10^8$</td>
<td>Inflammatory reaction at the disease site; Fever and tremors at the 2\textsuperscript{nd} infusion</td>
<td>PR (8 months)</td>
</tr>
<tr>
<td>3.JW</td>
<td>$5.2 \times 10^8$</td>
<td>None</td>
<td>PD</td>
</tr>
<tr>
<td>4.ST</td>
<td>$14 \times 10^8$</td>
<td>None</td>
<td>SD (8 months)</td>
</tr>
<tr>
<td>5.GG</td>
<td>$5.6 \times 10^8$</td>
<td>None</td>
<td>PD</td>
</tr>
<tr>
<td>6.PC</td>
<td>$7.2 \times 10^8$</td>
<td>None</td>
<td>SD (11+ months)</td>
</tr>
<tr>
<td>7.VL</td>
<td>$9.6 \times 10^8$</td>
<td>Orbital oedema and visual field defects</td>
<td>PR (5 months)</td>
</tr>
<tr>
<td>8.MC</td>
<td>$8 \times 10^8$</td>
<td>None</td>
<td>MR (10+ months)</td>
</tr>
<tr>
<td>9.GFMB</td>
<td>$6.4 \times 10^8$</td>
<td>None</td>
<td>PD</td>
</tr>
<tr>
<td>10.MM</td>
<td>$7.2 \times 10^8$</td>
<td>None</td>
<td>PD</td>
</tr>
</tbody>
</table>
RESULTS (3): Immunological effects of CTL infusion - response to LMP2

![Graph showing spot-forming units/10^5 cells at baseline, post-lymphodepletion, and 1.5 mos post-CTL infusion for patients P1 to P10.]
RESULTS (3): Immunological effects of CTL infusion - response to LMP2

Spot-forming units/10^5 cells

Baseline
Post-lymphodepletion
+ 1.5 mos post-CTL infusion
CONCLUSIONS

- Feasible and well tolerated
  - No significant side effects from lymphodepleting chemotherapy and CTL infusion
- Clinical benefit observed in advanced-stage, chemo-refractory patients
- Response seems associated to an increase in the frequency of peripheral blood T-cells specific for EBV subdominant antigens expressed by the tumor
FUTURE DIRECTIONS

- Cell therapy with EBV-specific CTL earlier in the course of NPC disease
- Increasing the number of LMP2 and/or LMP1-specific T cells in the infusion product