Flow Cytometric Applications for Immunotherapy of Cancer

Bone Marrow Transplantation and Cellular Therapy Program and Divisions of Neuro-oncology and Neurosurgery
University of Alabama at Birmingham
Flow cytometry has evolved beyond immunophenotyping into a powerful tool for immunotherapy design and monitoring.
Flow Cytometric Immune Monitoring

- **Preparation:** Pre-treatment immune status – immunophenotyping, immune function, cytotoxicity
- **Weapons:** Graft qualification – composition and potency.
- **Surveillance:** Immunophenotyping, activation and cytotoxicity and minimal residual disease assessment (in hematopoietic malignancies).
MALIGNANT GLIOMA
Basic concepts

• Innate immune activation as cancer cells are stressed – a new hope?
• Chemotherapy, radiation, and tumor-derived immunosuppressive cytokines suppression – the empire strikes back.
• Immune reconstitution and immunotherapy strategies – return of the cancer-killing Jedi?
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
Gliomas express stress-associated antigens that are targets for NK and γδ T cells

From Friese MA et al
Cancer Res. 2003; 63: 8996-9006
Tumor-derived Immunosuppression and Immune Escape

- Down-regulation of MHC Class I
- Secretion of immunosuppressive proteins
- Induction of regulatory T cells (CD3+CD4+CD25+FoxP3+).
- MSC population of tumor stroma
- Interference with costimulatory factors such as down regulation of CD40L
- NK inhibitory receptors on MHC Class I- cells
αβ and γδ T cell phenotypes in GBM patients (Six-color immunophenotyping)
NK and $\gamma\delta$ T cell phenotypes in GBM patients
Circulating Regulatory T cells in Patients with Gliomas

A

Grade II  Grade III  Grade IV

B

% of CD4 CD25

Grade II (n=3)  Grade III (n=4)  Grade IV (n=12)
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Mitogen-stimulated γδ T cell proliferation in GBM patients
Flow Cytometric Tracking of Graft Manipulation and Purity

Pre-selection

Post selection
γδ T cells and αβ T cells vs. U251 Suspension cytotoxicity assay (4 hr)
γδ T cell cytotoxicity vs. primary GBM and GBM cell lines (PKH26/ToPro Iodide)
Cytotoxicity of GBM patient-derived γδ T cells vs. U251 (PKH26/ToPro Iodide)
U251\textsuperscript{luc} GBM Treated with allogeneic expanded/activated \(\gamma\delta\) T cells

Saline-injected control mice

\(\gamma\delta\)-injected mice (5:1)  

Week 2 post-injection
Effect of γδ T cells on Induction and Growth of U251ffLuc Intracranial Gliomas

U251ffLuc cells injected alone or with γδ T cells

- U251ffLuc Cells (2.4x10^5)
  MST = 21 days
- U251 Cells + γδ T cells (1.2x10^6)
  MST = 48 days

p < 0.001
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Post-BMT Immune Recovery
TCD vs. T Replete Matched Unrelated Grafts
Post-BMT Immune Recovery
TCD vs. T Replete Matched Unrelated Grafts
UPN 97 T CELL RECOVERY

![Graph showing T cell recovery over days post BMT with lines representing CD3, CD3+CD4+, and CD3+CD8+ cells.](image)

**Axes:**
- **X-axis:** Days post BMT
- **Y-axis:** % of total lymphocytes
T Cell Function 100 Days Following Allogenic TCD BMT
CD4+ T Cell Function in NSCLC Patients Following Induction Chemotherapy

2D Graph 1

- UPN 9622 (Pre-Chemo: 500, Post-Chemo: 100)
- UPN 10249 (Pre-Chemo: 500, Post-Chemo: 200)

Legend:
- Pre Chemo
- Post-Chemo
Flow Cytometric Immune Monitoring

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MRD Monitoring - Issues

• Requires highly trained technical staff familiar with multiparameter Boolean gating in flow cytometry analytical software.

• Best done on the same day as immune recovery testing to establish immune status and to plan for cellular therapy if warranted.

• Element of faith “evidence of things not seen”
**Case NL**  
**Amplification of VWF STR Locus**

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Richland Memorial Hospital  
Molecular Genetics Laboratory
**Case ES**
Amplification of CSFR STR Locus

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<th>Mixtures of Pre-Transplant Samples</th>
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<td>100 95 90 80 50 20 10 5 0</td>
</tr>
<tr>
<td>% Donor</td>
<td>0 5 10 20 50 80 90 95 100</td>
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</tbody>
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Post-Transplant Sample

Richland Memorial Hospital
Molecular Genetics Laboratory
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