Immunotherapy
Introduction

Immunotherapy

A type of therapy that supports the immune system to target specific types of cancer or boost the immune system.
Types of Immunotherapy

- Cytokines
- Treatment Vaccines
- BCG
- Monoclonal Antibodies
- Adoptive Cell Transfer
Cytokines
Small proteins made by cells that affect the behavior of other cells. Cytokines made by lymphocytes are called interleukins.
Treatment Vaccines

Vaccines made from the patient's own tumor cell that strengthens the body’s natural defenses against that cancer.
BCG

BCG is a live, attenuated/weakened strain of mycobacterium. It is an immunostimulant that is known to prevent the progression of human bladder carcinomas.
Monoclonal Antibodies

Antibodies gathered and cloned from a single mouse B-cell. Humanized to work in the human body. They bind specific targets in the body and causes the immune system to destroy the cancer cells
Adoptive Cell Transfer

When a patient's own immune cells (T-Cells) or immune cells from somebody else are taken out of the blood and then engineered to produce proteins that are specific receptors on the cell surface (chimeric antigen receptors). After the cells are altered they are put back into the body.
Immunosurveillance

Innate Natural Killer Cells and Humoral Cytotoxic T cells (CD8) are continuously surveying the periphery to detect pathogens transformed cells. They do this by monitoring somatic MHC-I proteins. Transformed cells could express a tumor specific peptide on their MHC-I complex. Mutation can also reactivate embryonic genes or over express self antigens. Both NK cells and Cytotoxic T cells express NKG2D receptors that recognize MHC proteins, T-cells also have T-Cell receptors that ligate MHC-1 complexes as well. Upon recognition of a the lymphocytes initiate cell mediated apoptosis. MHC-I are expressed by all somatic cells, MHC-II exclusive to antigen presenting cells. NK cells also have Fc receptors which recognize antibodies. Antibodies opsonize transformed cells for death.
Immunosurveillance
Recognition
Overexpression of self antigens.
Expression of embryonic antigens.
Changes in density of self antigens.
Interferon Alpha has been used to treat melanoma. Interferon Alpha increases NK cell proliferation. This treatment is typically used as an adjuvant therapy after removal of the tumor. In a randomized control study by the Eastern Cooperative Oncology Group, 287 patients were infused with large amounts of INF-alf over 48 weeks had significant prolongation of relapse free survival ($P=0.0023$, one tailed), the median disease free survival for the control group was 1 year compared to 1.7 for the experimental group. A significant median overall survival rate was observed ($P=0.0237$), the control group being 2.8 years and the experimental being 3.8. (Kirkwood et al.)

Kaposi sarcoma, and other hematologic cancers. INF-alf activates natural killer cells. Infusions with IL-2 stimulate T-Cell proliferation. A long term adjuvant melanoma study, between 1985 and 1993 Proleukin. A high dose of 600,00 IU/kg was administered intravenously for 15 minute intervals every 8 hours over five days. A second identical treatment was scheduled after 9 days of rest. This was repeated for 12 weeks. The results 6% complete responses, 10% partial responses in all sites of disease. Diseases did not progress in any patient responding more than 30 months. Atkins et al. concluded that this could benefit some patients with metastatic melanoma, especially in combination therapies.

Cytotoxic T cells (CD8) are the most effective anti tumor cell, followed by the Natural Killer Cell. NK cells express NKG2D receptor that recognizes MHC-1 proteins.
productive interaction between dendritic cell and $T_H$ cell

Figure 15.8 (part 2 of 2) The Biology of Cancer (© Garland Science 2014)
Naive T cell
moderate affinity
IL-2
γ
β

Activated T cell
high affinity
IL-2
γ
β
α
IL-2 receptor

Naive T cells express the low-affinity IL-2 receptor (IL-2Rβ and γ chains only)

T cell
low-affinity IL-2 receptor

IL-2
IL-2Rα

Activated T cells express the high-affinity IL-2 receptor (IL-2Rα, β, and γ chains) and secrete IL-2

Figure 8.12 (part 1 of 2) The Immune System, 4th ed. (© Garland Science 2015)
HPV

E6 and E7 destabilize p53 and Rb.

Demonstrated in vitro immortalization of keratinocytes has been demonstrated.

Thomas et al.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
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<tr>
<td>L1</td>
<td>Major capsid protein</td>
</tr>
<tr>
<td>L2</td>
<td>Minor capsid protein</td>
</tr>
<tr>
<td>E1</td>
<td>DNA helicase</td>
</tr>
<tr>
<td>E2</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>E4</td>
<td>Structural protein expressed by infected keratinocytes</td>
</tr>
<tr>
<td>E5</td>
<td>Cofactor for epidermal growth factor, regulates MHC class I expression</td>
</tr>
<tr>
<td>E6</td>
<td>p53 degradation, telomerase activation</td>
</tr>
<tr>
<td>E7</td>
<td>Binding to Rb, E2F release</td>
</tr>
</tbody>
</table>
Treatment Vaccines

In order for an adaptive response to initiate, the immune system must recognize the antigen. The process of central tolerance describes the apoptotic selection against self reactive T-Cells to prevent autoimmune disorders. CD8 cells will recognize cancer antigens such as mutated surface proteins or proteins that are expressed during embryonic tropoblasts (before central tolerance is established in the Thymus), or immature sperm proteins. There are about 100 Cancer Testis antigens that are markers for different types of cancers. It has been demonstrated in adjuvant clinical trials that injecting MAGEA (common melanoma CT antigen) can send tumors into regression. Over the course of two years patients were injected with recombined viral delivered MAGEA epitopes or synthetic versions, regression was observed in 20% of patients with 10% remission. During this study there was a high amount of MAGEA specific CD8 cells proliferating. All of the CT antigens heavily funded targets of research.
Figure 17.18 The Immune System, 4th ed. (© Garland Science 2015)
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Alternative name</th>
<th>Chromosome</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT1</td>
<td>MAGEA</td>
<td>X</td>
<td>11</td>
</tr>
<tr>
<td>CT2</td>
<td>BAGE</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>CT3</td>
<td>MAGEB</td>
<td>X</td>
<td>4</td>
</tr>
<tr>
<td>CT4</td>
<td>GAGE1</td>
<td>X</td>
<td>8</td>
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<tr>
<td>CT5</td>
<td>SSX</td>
<td>X</td>
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<td>CT6</td>
<td>NY-ESO-1 LAGE</td>
<td>X</td>
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<td>CT7</td>
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<td>SYPC1</td>
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<td>CT10</td>
<td>MAGEC2</td>
<td>X</td>
<td>1</td>
</tr>
</tbody>
</table>
The first FDA approved cancer sipuleucel-T (Provenge), which is a customized antigen extracted from an individual patient. The common prostate cancer antigen, prostatic acid phosphatase (PAP) is the target of this therapy. This is done through extraction of an antigen presenting dendritic cells where it is cultured and granulocyte-macrophage colony stimulating factor is added to the dendritic cells. The PAP-GM-CSF positive dendritic cells are then injected back into the patient where the immune system mounts a more aggressive response to transformed cells expressing PAP.
productive interaction between dendritic cell and $T_H$ cell
In this phase 2 trial it was determined that if at least 15% of the patients received benefit from the treatment, it was worth pursuing further studies of efficiency.

Two patients exhibited a 25-50% transient decrease in PSA. One patient experienced an increased PSA, followed by a drop to undetectable levels for 52 months.

However PSA levels in all but four of the patients increased.

Burch et al.
BCG

BCG is alive, attenuated/weakened strain of mycobacterium that is obtained from cows. BCG was originally formulated to prevent or treat tuberculosis. TB shots containing BCG are still given in some countries.

For cancer patients, BCG is an immunostimulant that is known to prevent the progression of human bladder carcinomas at its early stage.
BCG

BCG is a shot that works by attracting immunocytes. Some specific immunocytes it attracts include the lymphocytes CD4+TH and CD8+TC, macrophages, and NK cells.

These immunocytes gather in the cells and cause inflammation. The mechanisms for BCG anti-tumor action are unknown.

Few side effects: redness, swelling, mild pain, ulcer at the injection site.
Bladder cancer cells infected with BCG (shown in green), an effective treatment for early-stage bladder cancer. The same genetic mutations that cause bladder cancer also activate a mechanism in the cells that allows BCG to enter and destroy them.
Effects of BCG when used in preventing the progression of human bladder carcinomas

Red Line (surgery): There is more recurrence in those not treated with BCG.

Blue Line (surgery + BCG): There is less recurrence in those treated with BCG. Some were still recurrence-free after 5 years.
BCG Solution with or without PANVAC in Treating Patients with Localized Bladder Cancer

Treatment

Randomized Phase II clinical trial

Trial ID: 14-C-0036
NCI-2014-00115, 1310-1269, 140036, RD-13-X-04, 9539, NCT02015104

The purpose of this clinical trial is to find out if Poxvirus vaccine/PANVAC + Bacillus Calmette-Guerin/BCG solution is more effective than BCG alone in treating non-muscle invasive high-grade urothelial carcinoma of the bladder in patients who have failed to respond to intravesical BCG within 1 year post treatment.
Monoclonal Antibodies

They are derived from a single B-cell clone

Monoclonal antibodies are targeted therapy

They help fight cancer by binding specific targets in the body and marking them

This causes the immune system to destroy the cancer cells
Making Monoclonal Antibodies

1a A mouse is injected with a foreign protein (antigen).

2 The mouse’s B-cells produce an antibody to recognize the antigen.

3 A few days later, antibody-producing B-cells are taken from the mouse’s spleen.

4 The mouse cells and tumor cells are mixed together in suspension.

5 Some of the mouse cells fuse with tumor cells to make hybrid cells called hybridomas.

6 The mixture of cells is placed in a selective medium that allows only hybrid cells to grow.

7 Hybridomas are screened for antibody production. They are then cultured to produce large numbers of monoclonal antibodies.

1b Pure tumor cells are harvested from culture.
Types of monoclonal antibodies

- Naked
- Conjugated
- Bispecific
Naked Monoclonal Antibodies

They are the most common type of mAbs used to treat cancer

They work by themselves meaning that they do not have a drug or radioactive material attached to them

Most naked mAbs attach to antigens on cancer cells

Some bind to antigens on other, non-cancerous cells, or even free-floating proteins
Naked Monoclonal Antibodies

The best known passive immunization treatment involves the monoclonal antibody Herceptin/trastuzumab.

Herceptin works best with the EGF receptor-related protein HER2/Neu.

HER2/Neu is over expressed in cancer cells due to amplification of the receptor-encoding gene.

HER2/Neu is not a tumor-specific antigen but does allow for Herceptin to preferentially kill the tumor cells.

Herceptin can stay in the body for about a month.
MM-302 Plus Trastuzumab vs. Chemotherapy of Physician's Choice Plus Trastuzumab in HER2-Positive Locally Advanced/Metastatic Breast Cancer Patients

Phase III, Phase II

Biomarker/Laboratory analysis, Treatment

Trial ID’s: MM-302-02-02-03
NCI-2014-02093, NCT02213744

open label, randomized, multicenter trial of MM-302 plus trastuzumab
MM-302 Plus Trastuzumab vs. Chemotherapy of Physician's Choice Plus Trastuzumab in HER2-Positive Locally Advanced/Metastatic Breast Cancer Patients

Purpose is to test if MM-302 + trastuzumab is more effective than the chemotherapy of physician's choice (CPC) + trastuzumab in locally advanced/metastatic

It is tested in HER2-positive breast cancer patients who have received prior treatment with trastuzumab in any setting and who have either progressed or are intolerant to each of pertuzumab and ado-trastuzumab emtansine in the metastatic or locally advanced setting and were not previously treated with an anthracycline in any setting.
Conjugated Monoclonal Antibodies
(Also known as tagged, labeled, loaded antibodies)

joined to a chemotherapy drug or to a radioactive particle

used as a homing device to take one of these substances directly to the cancer cells

circulates throughout the body until it can find and hook onto the target antigen.
delivers the toxic substance where it is needed most.

lessens the damage to normal cells in other parts of the body
Conjugated Monoclonal Antibodies

Brentuximab vedotin (Adcetris®)

an antibody that targets the CD30 antigen (found on lymphocytes), attached to a chemo drug called MMAE. This drug is used to treat Hodgkin lymphoma and anaplastic large cell lymphoma.
Bispecific Monoclonal Antibodies

made up of parts of 2 different mAbs

can attach to 2 different proteins at the same time
Bispecific Monoclonal Antibodies

example is blinatumomab (Blincyto)

used to treat some types of acute lymphocytic leukemia (ALL).

One part of blinatumomab attaches to the CD19 protein, which is found on some leukemia and lymphoma cells. Another part attaches to CD3, a protein found on immune cells called T cells. By binding to both of these proteins, this drug brings the cancer cells and immune cells together, which is thought to cause the immune system to attack the cancer cells.
Possible side effects of monoclonal antibodies

Fever
Chills
Weakness
Headache
Nausea

Vomiting
Diarrhea
Low blood pressure
Rashes
Evidence: IS Role in Controlling Tumor Growth

1- Increased frequency of cancer in patients with immune deficiencies

2- Tumors often evolve to become less visible to the IS
Adoptive T Cell Therapy

“Effective cancer immunotherapy is dependent on the presence of large numbers of anti-tumor lymphocytes with appropriate homing and effector functions that enable them to seek out and destroy cancer cells in vivo”

Immunotherapy in which anti-tumor lymphocytes are identified and grown ex vivo and then infused into the cancer patient, often along with vaccines or growth factors
Why ACT

1- Infusion of ex vivo cultured lymphocytes allows large number of anti-tumor lymphocytes can be generated and infused.

2- Capability to evaluate the action of cells prior to infusion allows to identify highly selected cells with high avidity for tumor antigens

3- It provide ability to manipulate the host before infusion to provide a better environment for the transferred cells.
How ACT Works

- 1987 TIL could grow in IL-2
- Initial clinical trial on melanoma patient: <0.01% of cell were in circulation only after a week
- Need for lymphodepletion
Lymphodepletion

- T cells, B cells and NK cells compete for homeostatic and activating cytokines such as IL-2, IL-7 and IL-15.
- Treg and NK cells and macrophages display suppressive activity by direct cell-to-cell contact or release of suppressive cytokines.
- Immature DCs might fail to activate or might even anergize ATC, limiting the antitumor response.
- The tumor acts as a negative regulator of T-cell activation by actively producing regulatory molecules and by expressing only limited numbers of MHC, rendering tumor recognition difficult.
## Preparative Regimens for Cell Transfer

<table>
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<th>Days</th>
<th>-7</th>
<th>-6</th>
<th>-5</th>
<th>-4</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
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<th>2</th>
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<tr>
<td><strong>Non-myeloablative</strong></td>
<td>Cy</td>
<td>Cy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>Cells</td>
<td>IL-2</td>
<td>IL-2</td>
<td>IL-2</td>
</tr>
<tr>
<td><strong>Ablative</strong> (200cGy)</td>
<td>Cy</td>
<td>Flu</td>
<td>Cy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>TBI</td>
<td>Cells</td>
<td>IL-2</td>
<td>IL-2</td>
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<tr>
<td></td>
<td>Flu</td>
<td>CD34+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IL-2</td>
<td></td>
</tr>
<tr>
<td><strong>Ablative</strong> (1200cGy)</td>
<td>Cy</td>
<td>Flu</td>
<td>Cy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>TBI</td>
<td>TBI</td>
<td>TBI</td>
<td>Cells</td>
<td>IL-2</td>
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<tr>
<td></td>
<td>Flu</td>
<td>CD34+</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>IL-2</td>
<td>CD34+</td>
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</table>
# Clinical Trial

Table 2. Frequency and Duration of Objective Responses

<table>
<thead>
<tr>
<th>TBI</th>
<th>Total No. of Patients</th>
<th>No.</th>
<th>%</th>
<th>PR Duration (months)</th>
<th>CR No.</th>
<th>%</th>
<th>Duration (months)</th>
<th>OR No.</th>
<th>%</th>
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<tbody>
<tr>
<td>None*</td>
<td>43</td>
<td>17</td>
<td>39.5</td>
<td>64+, 32+, 20+, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2</td>
<td>4</td>
<td>9.3</td>
<td>63+, 58+, 48+, 47+</td>
<td>21</td>
<td>48.8</td>
</tr>
<tr>
<td>2 Gy</td>
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<td>33+, 29+, 23+, 14, 10, 6, 5, 5, 4, 3, 3</td>
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<td>8.0</td>
<td>37+, 25+</td>
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<td>12 Gy</td>
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<td>16.0</td>
<td>17+, 15+, 13+, 8+</td>
<td>18</td>
<td>72.0</td>
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</table>

NOTE: All patients received cyclophosphamide 60 mg/kg × 2 days + fludarabine 25 mg/m² × 5 days.
Abbreviations: TBI, total-body irradiation; PR, partial response; CR, complete response; OR, objective response; TIL, tumor-infiltrating lymphocytes.
Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

Proportion Surviving

Survival Time in Months

(TBI 1200: n=25)

(TBI 200: n=25)

(NMA: n=43)

(No lymphodepleting regimen: n=86)
Objective response rates (RECIST) in metastatic melanoma patients treated in the Surgery Branch, NCI
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Primary Literature


