Causes of Death after Autologous HCT done in 2013-2014

- Primary Disease: 69%
- Infection: 24%
- Organ Failure: 2%
- Second Malignancy: 2%
- Other: 3%
Causes of Death after HLA Matched Sibling HCT done in 2013-2014

Died within 100 days post-transplant
- Primary Disease: 36%
- GVHD: 29%
- Infection: 9%
- Organ Failure: 9%
- Other: 16%
- Hemorrhage: 1%

Died at or beyond 100 days post-transplant
- Primary Disease: 25%
- GVHD: 57%
- Infection: 7%
- Organ Failure: 7%
- Other: 3%
Causes of Death after Unrelated Donor HCT done in 2013-2014

Died within 100 days post-transplant

- Primary Disease: 34%
- Graft Rejection: 11%
- Infection: 18%
- Hemorrhage: 2%
- Organ Failure: 10%
- GVHD: 2%
- Other: 1%

Died at or beyond 100 days post-transplant

- Primary Disease: 27%
- Graft Rejection: 10%
- Infection: 9%
- Hemorrhage: 6%
- Organ Failure: 1%
- GVHD: 1%
- Secondary Malignancy: 1%
- Other: 1%

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Hematopoietic Cell Transplantation

Donor hematopoietic cells
(ideally MHC matched)

Conditioning regimen

GVL

T cells

GVHD
What is Graft versus Host Disease ("GVHD")?

- Syndrome observed in recipients of allogeneic hematopoietic cells
- Transplanted immune cells attack recipient’s ("host") cells
- Symptoms may include:
  - Fever
  - Rash
  - Nausea
  - Vomiting
  - Abdominal pain
  - Diarrhea
  - Eye irritation
  - Immune-mediated pneumonitis
  - Damage to connective tissue and exocrine glands
  - Sloughing of mucosal membrane
- Can be life threatening or fatal
- Treatment based on glucocorticoids such as prednisone, and other agents
Defining the GVL effect: GVHD and relapse after HLA-matched allogeneic BMT

Horowitz, M et al., Blood 75: 555-562 (1990)
Timeline showing numbers of bone marrow transplants and advances in the field, 1957-2006

The laws of histocompatibility in allogeneic HCT

1. Donor/recipient genetic non-identity for the HLA antigens encoded in the MHC is the single most important risk factor for the development of severe GVHD.

2. Clinically significant GVHD – that can sometimes be life-threatening or even fatal – still occurs in recipient of hematopoietic cell grafts from HLA-identical donors.

WHY?
What is involved in HLA typing, anyway?

- **HLA-A**
- **HLA-C**
- **HLA-B**
- **HLA-DRB1**
- **HLA-DQB1**
- **HLA-DPB1**

**6p21.3**

- Chromosome 6

**Mb**

- 29.0
- 30.0
- 31.0
- 32.0
- 33.0
- 34.0

- **Exons 2 and 3 in each gene**

- **TOTAL**: 1,925 bp

- **Exon 2**

- **Exon 2**

- **7 exons total**
- ~275 nt each

- **Haploid genome**: 3.3 x 10^9 bp

- < 1 x 10^{-3} of the MHC

- < 1 x 10^{-6} of the haploid genome
T cells recognize short peptides presented on the cell surface by MHC molecules.

CD8⁺

NY-ESO-1₁₅₇-₁₆₅
HLA-A*0201

CD4⁺

MBP₂₁₇-₂₃₀
HLA-DR2
These 1,925 bp encode the peptide binding regions of class I and II MHC molecules.

Minor antigen HA-1
VLHDDDLLEA
HLA-A*02:01:01

Influenza A HA peptide
PKYVKQNTLKLAT
HLA-DR4
The composition of the MHC-associated peptidome defines the immunologic identity of a cell

~100,000 MHC class I molecules/cell (perhaps $1 \times 10^6$...)

MHC class II molecules on APC’s

~10,000 distinct peptides derived from:

- nuclear proteins
- cytoplasmic proteins
- membrane proteins
- cryptic proteins...

Unexpected translation products; spliced peptides
The composition of the MHC-associated peptidome is genome- and tissue-specific and very dynamic.
Schematic overview of the cellular and molecular mediators, known and implicated, contributing to the continuum of acute GVHD and chronic GVHD pathology.
Major sites of acute graft-versus-host disease

Skin

GI Tract

Liver
Initiation phase of acute GVHD

**TRIGGERS**
- Histocompatibility disparities (HLA, miHA, KIR)
- Danger signals (PAMPs and DAMPs)

**SENSORS**
- Host non-hematopoietic APCs
- Host DCs, Langerhans, B cells
- Donor hematopoietic APCs

**Initiation phase**
- TNF-a
- IL-1b
- IL-6
- Others

**Cytokine Response**
- Host APC activation

**APC presentation of Ag to T cells**

**Recruitment of effectors**

**Shernan G. Holtan et al. Blood 2014;124:363-373**

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Expansion, trafficking, and effector phase of acute GVHD

- Trafficking, expansion, and effector phase
- Suppressors reduce GVHD
  - Tregs
  - NK and NKT cells
  - MDSC
  - B cells
- Naïve T cells
- Memory T cells
- NK and NKT cells
- B cells
- Activated macrophages
- Th1, Th2, Th17 cytokines
- Effectors and amplifiers increase GVHD
- Overall balance relates to extent of organ damage: skin, gut, liver, endothelium
- Biomarkers: Elafin, REG3a, ST2, miRNA
Treatment phase of acute GVHD

**Resolution**
- Complete repair of target organ damage
- Good graft function
- Immune reconstitution

**Persistent inflammation**
- Delayed immune recovery, “injurious resolution”
- Mild to moderate cytopenias
- Chronic GVHD

**Progression**
- Steroid refractoriness or high-dose dependence
- Mild to severe cytopenias
- Markedly impaired immune function, high risk of early death

## Approaches to prevention and treatment of acute GVHD

### Table 1. Emerging approaches for the prevention and treatment of aGVHD

<table>
<thead>
<tr>
<th>Treatment or pathway</th>
<th>Potential mechanism(s) of action</th>
<th>Level of evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small molecules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKC inhibitors, such as R524 (Rigel Pharmaceuticals), and sotrasaurin (Novartis)</td>
<td>Inhibition of PKCα/θ, proteins that maintain immunologic synapse between APC and effector T cell</td>
<td>Preclinical (mouse) Sotrasaurin being investigated in solid organ transplant clinical trials</td>
<td>125,126</td>
</tr>
<tr>
<td>Sphingosine 1-phosphate receptor agonist</td>
<td>Modulates DC function and lymphocyte efflux from secondary lymphoid organs, enhancement of endothelial barrier function</td>
<td>Preclinical (mouse)</td>
<td>127</td>
</tr>
<tr>
<td>FTY720 (Fingolimod; Gilenia Pharmaceuticals)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypomethylating agents azacitidine and decitabine</td>
<td>Induction of FOXP3 expression</td>
<td>Preclinical (mouse) phase 1/2 clinical</td>
<td>128,129</td>
</tr>
<tr>
<td><strong>Retinoic acid signaling</strong></td>
<td>Reduction of T-cell homing, reducing Th1 differentiation, inducing Tregs</td>
<td>Preclinical (mouse)</td>
<td>130</td>
</tr>
<tr>
<td><strong>Tim-3/Gal-9 pathway</strong></td>
<td>Increased activation-induced T-cell death in the absence of Tregs</td>
<td>Preclinical (mouse)</td>
<td>131</td>
</tr>
<tr>
<td><strong>PDL-1 pathway</strong></td>
<td>Coinhibitory molecule, conversion of Th1 cells to Tregs</td>
<td>Preclinical (mouse)</td>
<td>132</td>
</tr>
<tr>
<td><strong>IDO</strong></td>
<td>Rate-limiting enzyme in tryptophan (required for T-cell proliferation) metabolism</td>
<td>Preclinical (mouse, human)</td>
<td>133,134</td>
</tr>
<tr>
<td><strong>Arginase-1</strong></td>
<td>L-arginine depletion, reducing T-cell signaling and inflammatory cytokines</td>
<td>Preclinical (mouse)</td>
<td>59</td>
</tr>
<tr>
<td><strong>TLR/MyD88 signaling inhibitors</strong></td>
<td>Interfere with danger signaling, especially via inhibitory oligonucleotides against TLR9, to reduce inflammation</td>
<td>Preclinical (mouse)</td>
<td>135</td>
</tr>
<tr>
<td><strong>Notch/notch ligand inhibitors</strong></td>
<td>Detal-like1/4 (notch ligand) inhibitor given peritransplant prevented GVHD, while Notch 1 inhibitor lead to intestinal toxicity</td>
<td>Preclinical (mouse)</td>
<td>136</td>
</tr>
</tbody>
</table>
### Approaches to prevention and treatment of acute GVHD (2)

<table>
<thead>
<tr>
<th>Cytokine/growth factor modulation</th>
<th>Summary</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK/STAT inhibition</td>
<td>Reduction in inflammatory cytokines</td>
<td>Preclinical, case report</td>
</tr>
<tr>
<td>IL-17 downregulation</td>
<td>Curcumin downregulates IFN-γ and IL-17 production, ameliorating aGVHD</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>IL-21 blockade</td>
<td>Enhances generation of inducible Tregs</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>IL-22 augmentation</td>
<td>Protective factor for intestinal stem cells under immune attack</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>IL-23 blockade</td>
<td>Reduces inflammatory cytokines, T-cell trafficking, gut protection</td>
<td>Preclinical (mouse); Case report, phase 2 (ongoing)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell-based therapies</th>
<th>Summary</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCs</td>
<td>Suppress immune effector functions, secrete cytokines/growth factors for tissue repair and angiogenesis, can be obtained from related donors or third party</td>
<td>Phase 3, not yet reported in peer-reviewed literature (NCT00366145)</td>
</tr>
<tr>
<td>MAPCs</td>
<td>No expression of classical HLA class I markers (distinct from MSC), suppress T-cell activation via prostaglandin E2 synthesis, but only if colocalized with T cells at sites of activation</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>Tregs</td>
<td>Expanded from umbilical cord blood, reduced aGVHD grade II-IV incidence from 61% to 43% in double UCB HCT (historical control); in haploidentical-related donors, Tregs reduced GVHD and enhanced immune reconstitution</td>
<td>Phase 1</td>
</tr>
<tr>
<td>TRAIL⁺ T cells</td>
<td>Cytolytic mechanism against both tumor cells and alloreactive T cells</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>NKs</td>
<td>GVHD protection only conferred if infusion was derived from Ly49-mismatched donor</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>NKTs</td>
<td>Invariant NKTs attenuated murine GVHD in association with increased IL-2, IL-4, and IL-5 levels</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>DCs</td>
<td>Tolerogenic DCs enhanced immunosuppressive cytokines in circulation, increased Tregs</td>
<td>Preclinical (mouse)</td>
</tr>
<tr>
<td>MDSCs</td>
<td>L-arginine depletion, contact-dependent immunosuppression</td>
<td>Preclinical (mouse)</td>
</tr>
</tbody>
</table>

IDO, indoleamine 2,3 dioxygenase; IFN, interferon; JAK, Janus kinase; KGF, keratinocyte growth factor; MAPC, multipotent adult stromal cell; PDL, programmed death ligand; PKC, protein kinase C; STAT, signal transducer and activator of transcription; TRAIL, TNF-related apoptosis inducing ligand; UCB, umbilical cord blood.
Approaches to prevention and treatment of acute GVHD (3)

<table>
<thead>
<tr>
<th>Treatment or pathway</th>
<th>Potential mechanism(s) of action</th>
<th>Level of evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiota</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-defensins</td>
<td>Antimicrobial peptides secreted by intestinal Paneth cells, a target of GVHD</td>
<td>Preclinical (mouse)</td>
<td>151</td>
</tr>
<tr>
<td>Physiologic diversity</td>
<td>GVHD causes increase in Lactobacillales and decreases in Clostridiales, resulting in loss of physiologic diversity in gut bacteria</td>
<td>Preclinical (mouse and human)</td>
<td>152</td>
</tr>
<tr>
<td><em>Candida</em> colonization</td>
<td>Patients colonized with <em>Candida</em> spp. had an increased incidence of grade II-IV GVHD (50% vs 32%)</td>
<td>Preclinical (human)</td>
<td>153</td>
</tr>
<tr>
<td>α-galactosylceramide (RGI-2001; Regimmune)</td>
<td>Produced by microbiome, can bind C1d and activate NKTs, induce Tregs</td>
<td>Preclinical (mouse)</td>
<td>154,155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase 1/2a (ongoing)</td>
<td></td>
</tr>
<tr>
<td><strong>Donor-based immunomodulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KGF (palifermin)</td>
<td>Epithelial, including thymic cytoprotection, inflammatory cytokine response, skewing toward Th2 cytokine response, although there was no reduction in GVHD when recipients were treated with palifermin in a phase 1/2 clinical trial</td>
<td>Preclinical (mouse)</td>
<td>156,157</td>
</tr>
<tr>
<td>Statins</td>
<td>Retrospective study demonstrated reduced grade III-IV GVHD in related HCT from statin-treated donors</td>
<td>Preclinical (mouse, human)</td>
<td>Phase 2 (ongoing)</td>
</tr>
</tbody>
</table>

IDO, indoleamine 2,3 dioxygenase; IFN, interferon; JAK, Janus kinase; KGF, keratinocyte growth factor; MAPC, multipotent adult stromal cell; PDL, programmed death ligand; PKC, protein kinase C; STAT, signal transducer and activator of transcription; TRAIL, TNF-related apoptosis inducing ligand; UCB, umbilical cord blood.
Overall survival after diagnosis of acute GVHD, by grade

Acute, late acute, chronic overlap, and classic chronic GVHD
The frequency of involvement by chronic GVHD varies across organs and sites and is higher after HCT with mobilized blood cells as compared with marrow.

Mary E. D. Flowers, and Paul J. Martin *Blood* 2015;125:606-615
Skin and oral manifestations of chronic GVHD

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Stephanie J. Lee Blood 2017;129:30-37
Diagnosis of chronic GVHD, according to the NIH consensus criteria

- Clinical sign(s) compatible with GvHD
  - Chronic GvHD
    - Skin (lichenoid, sclerotic...)
    - Mouth
    - Nails and hair
    - Eyes
    - Lung
    - Musculoskeletal
    - Hematopoietic
    - Gastro-intestinal (esophageal)
    - Liver
    - Other
  - Acute GvHD
    - Skin
    - Gastro-intestinal
    - Liver
  - Subsequent episode
    - Recurrent
    - Persistent
    - New Onset or late acute
  - 1st episode
    - Classical

- Any sign of Acute GvHD?
  - No
    - Classical Chronic GvHD
  - Yes
    - Overlap Syndrome

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Gérard Socié and Jerome Ritz Blood 2014;124:374-384
### Severity of chronic GVHD: mild, moderate, severe

<table>
<thead>
<tr>
<th>Severity</th>
<th>Description</th>
</tr>
</thead>
</table>
| Mild       | - 1 or 2 organs or sites (except lung) with score 1  
            |   - Mild oral symptoms, no decrease in oral intake  
            |   - Mild dry eyes, lubricant eyedrops \( \leq 3x/\text{day} \)  |
| Moderate   | - 3 or more organs with score 1  
            |   - At least 1 organ or site with score 2  
            |   - 19-50% body surface area involved or superficial sclerosis  
            |   - Moderate dry eyes, eyedrops \( > 3x/\text{day} \) or punctal plugs  
            |   - Lung score 1 (FEV1 60-79% or dyspnea with stairs)  |
| Severe     | - At least 1 organ or site with score 3  
            |   - > 50% body surface area involved  
            |   - Deep sclerosis, impaired mobility or ulceration  
            |   - Severe oral symptoms with major limitation in oral intake  
            |   - Severe dry eyes affecting ADL  
            |   - Lung score 2 (FEV1 40-59% or dyspnea walking on flat ground)  |
Mechanistic interventions for the prevention or treatment of chronic GVHD

**Stem cell graft engineering**
- Anti-thymocyte globulin
- Post-transplant cyclophosphamide
- CD34 selection
- Ex vivo pan-T cell depletion
- Ex vivo selective T cell depletion
- Donor IL-2 therapy

**Inhibit T cell signaling**
- ITK inhibition - ibrutinib
- JAK1/2 inhibition - ruxolitinib
- ROCK2 inhibition - KD025
- bortezomib

**Adoptive Treg Therapy**
- Purified donor Treg
- Ex vivo expanded Treg
- Antigen-specific Treg

**CD4⁺ FoxP3⁺ Regulatory T cells**

**Treg-sparing therapy**
- sirolimus
- mycophenolate mofetil
- ruxolitinib
- bortezomib

**In vivo Treg expansion**
- ECP
- low-dose IL-2

**B cell depletion in vivo**
- rituximab
- ofatumumab
- obinutuzumab

**Inhibit B cell signaling**
- BTK inhibition - ibrutinib
- SYK inhibition - fostamatinib

Corey S. Cutler et al. Blood 2017;129:22-29
Prophylactic regimens and treatments for chronic GVHD

<table>
<thead>
<tr>
<th>Randomized trial</th>
<th>Main results on chronic GVHD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute GVHD prophylaxis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitors +/- methotrexate</td>
<td>No effect</td>
<td>10, 76</td>
</tr>
<tr>
<td>Prolonged cyclosporine</td>
<td>No effect</td>
<td>10, 76</td>
</tr>
<tr>
<td>Ex vivo T-cell depletion</td>
<td>No effect</td>
<td>10, 76, 77</td>
</tr>
<tr>
<td>Antithymocyte globulin</td>
<td>Decreased incidence</td>
<td>72-75</td>
</tr>
<tr>
<td><strong>First-line treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone +/- azathioprine</td>
<td>Prednisone better</td>
<td>79</td>
</tr>
<tr>
<td>Prednisone + cyclosporine vs prednisone</td>
<td>Reduced prednisone exposure with combination</td>
<td>80</td>
</tr>
<tr>
<td>Prednisone + cyclosporine vs prednisone + cyclosporine + thalidomide</td>
<td>No benefit of thalidomide</td>
<td>81, 82</td>
</tr>
<tr>
<td>Prednisone + cyclosporine vs prednisone + cyclosporine + mycophenolate mofetil</td>
<td>No benefit of mycophenolate mofetil</td>
<td>83</td>
</tr>
<tr>
<td>Prednisone + cyclosporine vs prednisone + cyclosporine + hydroxychloroquine</td>
<td>No benefit of hydroxychloroquine</td>
<td>84</td>
</tr>
</tbody>
</table>
Second-line treatment of chronic GVHD

A

- Extracorporeal photopheresis
- Mycophenolate mofetil
- Thalidomide
- Sirolimus or everolimus
- Rituximab
- Pentostatin
- Methotrexate
- Tacrolimus
- Imatinib
- Thoraco-abdominal irradiation
- Pulse steroid
- Mesenchymal stem cells
- Etretinate
- Etanercept
- Clofazimine
- Chloroquine
- Alefacept

Number of reports

B

- Successful withdrawal of IST
- Relapse + NRM + Treatment change

Percent

Months from Second-line Treatment

- Relapse
  - 3%
  - 4%
  - 4%
  - 5%
  - 5%
  - 5%
  - 5%
  - 5%
  - 5%
- NRM
  - 7%
  - 9%
  - 11%
  - 11%
  - 11%
  - 11%
  - 11%
- Treatment change
  - 34%
  - 43%
  - 50%
  - 53%
  - 55%
  - 57%
  - 59%
- FFS
  - 55%
  - 45%
  - 35%
  - 31%
  - 29%
  - 28%
  - 26%
  - 25%
- Withdrawal of IST
  - 1%
  - 2%
  - 5%
  - 8%
  - 11%
  - 13%
  - 14%
  - 15%

Gérard Socié and Jerome Ritz Blood 2014;124:374-384
• https://www.cibmtr.org/pages/index.aspx
• https://momentum.vicc.org/2016/06/the-mds-challenge/