The Villain Returns: Disease Relapse Following Transplant, MRD Assessment and Treatment Strategies

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Veronika Bachanova, MD, PhD
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November 9, 2018
## Disclosures

The following faculty and planning committee staff have the following financial disclosures:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Disclosure</th>
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<tr>
<td>Alan Howard, PhD</td>
<td>CIBMTR</td>
<td>Jazz Pharmaceuticals, Travel/Lodging, Consultant</td>
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<tr>
<td>Misty Evans, DPN</td>
<td>Vanderbilt</td>
<td>Jazz Pharmaceuticals, Monetary, Speakers Bureau</td>
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<td>Veronika Bachanova, MD, PhD</td>
<td>University of Minnesota</td>
<td>None</td>
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<tr>
<td>Philip McCarthy, MD</td>
<td>Roswell Park Comprehensive Cancer Center</td>
<td>Celgene, Honoraria, Advisory Board, Karyopharm, Honoraria, Advisory Board, Celgene, Institute research Support, Research, Medscape, Honoraria, Generating content for online lecture, Axis, Honoraria, Generating content for MM lecture</td>
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Treating Disease Relapse after Allogeneic Hematopoietic Stem Cell Transplantation

• 1975 NEJM review of BMT - E. Donnall Thomas (BMT pioneer & Nobel Prize laureate) noted that one of the major barriers to the successful application of BMT was: “Relapse of Disease”.

• State of the Science Symposium – FEB 2014

   High Priority Trial Categories

   • Prevention of Post-Transplant Relapse
   • Application of HCT to Selected Non-Malignant Diseases
   • Prevention and Treatment of GVHD
   • Avoidance of HCT Complications

Grab your cape.
Learning objectives

• At the conclusion of this session, attendees will be able to:

  • Discover the incidence and continuing challenges of hematopoietic malignancy relapse following allogeneic hematopoietic stem cell transplantation.
  • Compare innovative methodologies to detect pre- and post-HCT minimal residual disease (MRD).
  • Analyze the promise of innovative cellular therapeutic strategies to treat and prevent relapse in HCT patients.
  • Evaluate the strategies employed when utilizing new targeted immunotherapeutic approaches to treat disease relapse.
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Minimal Residual Disease (MRD) Testing and Prevention of Relapse in Multiple Myeloma

AML and ALL Relapse Following HCT and Treatment Strategies

Grab your cape.
Minimal Residual Disease (MRD) Testing and Prevention of Relapse in Multiple Myeloma

Philip McCarthy
Roswell Park Comprehensive Cancer Center
Buffalo, NY
November 2018
The presentation will discuss off-label use of drugs for multiple myeloma treatment

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<td>Scientific Advisory Board</td>
<td>No relevant conflicts of interest to declare</td>
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Minimal Residual Disease Testing

• After Primary Therapy
  – Biomarker for prognosis
• At time points during follow-up/maintenance
  – Biomarker for prognosis
  – Endpoint for stopping or continuing therapy?
• Monitor for relapse
• Trial Design
  – Patient Stratification
  – Criterion for randomization to continued therapy or stopping therapy
  – Risk assessment for treatment arm selection
  – Can MRD serve as a surrogate endpoint for PFS/OS?
Treatment for the Transplant Eligible Newly Diagnosed Multiple Myeloma (NDMM) Patient

• Autologous Stem Cell Transplant (ASCT) after induction therapy
  – Standard for NDMM patient even with novel drug availability

• Maintenance +/-consolidation therapies post ASCT
  – Lenalidomide\(^1-3\) (Len) and bortezomib\(^4\) maintenance prolong response and Len maintenance improves overall survival\(^2,5-7\)

• However the majority of patients will have relapse/progression of disease
  – Continue to test new strategies to improve outcome
  – Add to standard maintenance therapy to improve outcome
  – *Early surrogate endpoints for long term outcome (PFS/OS) must be tested in clinical trials so as to prevent studies that must remain open for 10 years or longer especially for an OS endpoint (Examples include Minimal Residual Disease (MRD) testing and Immune Profiling)*

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Fractional Cell Kill and the Tip of the Iceberg (10%)

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https://en.wikipedia.org/wiki/Fractional_kill
https://commons.wikimedia.org/wiki/File:Iceberg.jpg
PFS: 14 studies (n=1273) & OS: 12 studies (n=1100) CR analysis: 5 studies (n=574) for PFS & 6 studies (n=616) for OS

PFS: MRD-negative, HR=0.41; 95% CI, 0.36-0.48; \( P < 0.001 \)

OS: MRD-negative, HR=0.57; 95% CI, 0.46-0.71; \( P < 0.001 \)

CR/PFS: MRD-negative, HR=0.44; 95% CI, 0.34-0.56; \( P < 0.001 \)

CR/OS: MRD-negative, HR=0.47; 95% CI, 0.33-0.67; \( P < 0.001 \)

MRD Assays: Multiparameter Flow Cytometry (10^{-4} to 10^{-6}) (n=9), Allele-specific oligonucleotide quantitative Polymerase Chain Reaction (10^{-4} to 10^{-6}) (n=11), Next Generation Sequencing (10^{-6}) (n=1)
Next Generation Sequencing (NGS): Technical principles

Sequenta Lymphosight, now Adaptive Biotechnologies

Functional Allele

Locus IGH 14q32

Non Functional Allele

Locus IGK 2p11

Courtesy H Avet-Loiseau
Blood 2017 130:435
NGS: Technical principles

FREQUENCY OF MYELOMA CLONE AMONG B CELLS = $S_l / (S_l + S_B)$

NUMBER OF MYELOMA MOLECULES PER LEUKOCYTE = $S_l \times (N_R/S_R) / N_{TOT}$

Courtesy H Avet-Loiseau
Blood 2017 130:435
FDA News Release September 28, 2018

FDA authorizes first next generation sequencing-based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma

The FDA granted marketing authorization of ClonoSEQ assay to Adaptive Biotechnologies

- Retrospective analysis of 3 previously conducted clinical studies
- ALL: 273 patients
- MM: 323 patients in an ongoing study and a study of 706 patients (IFM)
- ALL
  - ClonoSEQ assay assessed MRD at various disease burden thresholds
  - MRD level correlated with EFS
  - MRD negative, longer EFS and MRD positive, lower EFS
- MM
  - ClonoSEQ assay demonstrated similar associations with PFS and DFS

https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm622004.htm
Allele-specific oligonucleotide-quantitative PCR (ASO-qPCR) method to detect minimal residual disease (MRD), and design of ASO-qPCR primers and probes.

Takamatsu H, J Clin Med Oct 2017
# RPCC Comparison of MFC panels used for MRD testing over time

<table>
<thead>
<tr>
<th>Panel Years</th>
<th>Tube #</th>
<th>Monoclonal Antibody per Fluorochrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-2010</td>
<td>4 color, 6 mAb</td>
<td>FITC</td>
</tr>
<tr>
<td>2010-2014</td>
<td>4 color, 11 mAb</td>
<td>FITC</td>
</tr>
<tr>
<td>2014-2016</td>
<td>8 color, 10 mAb</td>
<td>FITC</td>
</tr>
</tbody>
</table>

1. **Panel A**
   - 2007-2010: 4 color, 6 mAb
   - 1 CD38, CD138, CD45, CD56
   - 2 CD38, cLambda, CD138, cKappa
   - # events / sensitivity: 2007-2010: <250,000 /10^-4

2. **Panel B**
   - 2010-2014: 4 color, 11 mAb
   - 1 CD38, CD10, CD19, CD34
   - 2 CD38, CD138, CD45, CD56
   - 3 CD38, CD117, CD45, CD28
   - 4 CD38, cLambda, CD138, cKappa
   - # events / sensitivity: 2010-2014: 250,000-1,000,000 /10^-4 - 10^-5

3. **Panel C**
   - 2014-2016: 8 color, 10 mAb
   - 1 CD38, CD56, CD45, CD19, CD117, CD81, CD138, CD27
   - 2 CD38, CD56, CD45, CD19, cKappa, cLambda, CD138, CD27
   - # events / sensitivity: 2014-2016: 1-6,000,000 /10^-5 - 10^-6

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1. PerCP; 2. Horizon V450; 3. LDAqua: Fixable Live Dead Aqua (viability)

Ammannagari et al ASH 2016, Abstract 2274; Manuscript in Preparation
Progression free survival (PFS) and overall survival (OS) according to MRD status by multiparameter flow cytometry at day +100 post-AHCT

Ammannagari et al. ASH 2016, Abstract 2274; Manuscript in Preparation

3-yr PFS 62% (95% CI: 52-72%) vs 33% (95% CI: 12-53%), P <0.0001
3-year OS 85% (95% CI: 78-93%) vs 64% (95% CI: 44-85%), P=0.004

Sensitivity at $10^{-5}$ to $10^{-6}$

N=172:
Progression free survival (PFS) and overall survival (OS) in patients who are MRD negative at day +100 post AHCT, stratified by numbers of analyzed plasma cells (PCN)

Ammannagari et al ASH 2016, Abstract 2274; Manuscript in Preparation

3-yr PFS at Day 100:
- PCN<250,000: 42% (95% CI: 20- 63%)
- PCN=250,000-1,000,000: 65% (95% CI 54-76%)
- PCN>1,000,000: 89% (CI 78-101%) (P=0.03)

N=172: Ammannagari et al ASH 2016, Abstract 2274; Manuscript in Preparation
Comparison of MSKCC single 10-color tube and EuroFlow two 8-color tubes.
% Abnormal PC

R² = 0.9736

The DETERMINATION Trial
IFM/DFCI 2009 Phase 3 Study
Newly Diagnosed MM (SCT candidates; n= originally 1000, now 1360)
Randomize

- **Induction**
  - RVDx3
  - CY (3g/m2) MOBILIZATION (Goal: 5x10^6 cells/kg)
  - Melphalan 200mg/m² + ASCT
  - RVD x 2
  - Lenalidomide

- **Consolidation**
  - RVDx3
  - CY (3g/m2) MOBILIZATION (Goal: 5x10^6 cells/kg)

- **Maintenance**
  - USA: until progression
  - IFM: for 1 year

- **Collection**
  - CY (3g/m2)
  - MOBILIZATION (Goal: 5x10^6 cells/kg)

- **SCT at relapse**
  - RVD x 5
  - Lenalidomide

**USA: 660 & IFM: 700 patients**

RVD=Revlimid®, Velcade®, dexamethasone. Cy=Cyclophosphamide. Courtesy P Richardson
Impact of cytogenetic risk?

Courtesy H Avet-Loiseau
Blood 2017 130:435
Impact of treatment arm?

A Progression-free Survival

B Overall Survival

Maintenance stopped

No. at Risk
RVD alone 350 294 228 157 32
Transplantation 350 308 264 196 50

No. at Risk
RVD alone 350 339 325 293 95
Transplantation 350 330 313 281 89

N at risk
positive MRD-Transplant 68 62 49 35 15 1
positive MRD-RVD 66 51 38 21 11 2
negative MRD_Transp 50 47 43 38 23 4
negative MRD_RVD 40 39 34 31 17 1

 Courtesy H Avet-Loiseau
Blood 2017 130:435
A: K-M Curves for PFS by MRD Status at the Start of Maintenance Therapy.
B: K-M Curves for PFS by MRD Status after 12 months of maintenance therapy.

Median PFS; MRD-negative patients: NR
Median PFS; MRD-positive patients: 29 months

Median PFS; MRD-negative patients: NR
Median PFS; MRD-positive patients: 20 months

A: K-M Curves for OS by MRD Status at the Start of Maintenance Therapy.
B: K-M Curves for OS by MRD Status after 12 months of maintenance therapy.

OS at 4 yr; MRD-negative patients: 94%
OS at 4 yr; MRD-positive patients: 79%

OS at 3 yr; MRD-negative patients: 96%
OS at 3 yr; MRD-positive patients: 86%

Perrot A et al Blood 2018, courtesy H Avet Loiseau
PFS/OS after PET CT normalization before Maintenance

Arm A (RVD x 8) PFS PET CT normalized vs positive
P=0.22

Arm B (RVD + ASCT) PFS PET CT normalized vs positive
P=0.004

Arm B (RVD + ASCT) OS PET CT normalized vs positive
P<0.001

PFS for PET CT normalized & MRD negative by flow cytometry before maintenance vs all others

Moreau et al JCO 35:2911, 2017
There is a 25% reduction in risk of death, representing an estimated 2.4-year increase in median survival (March 2015 data cutoff)\(^a\)

\(\text{HR (95\% CI, } P\text{-value)} = 0.75 (0.63-0.90) \times 0.001\)

**Overall Survival: Median Follow-Up of 80 Months**

**Hazard ratio for progression or death with lenalidomide maintenance, 0.47 (95\% CI, 0.33–0.65); } P<0.001**

**Time to Death by Cause of Death**

\(\text{HR, hazard ratio; maint, maintenance; IF, not reached; OS, overall survival}\

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\(\text{\(^a\) Log-rank test and Cox model stratified by study to assess impact of lenalidomide maintenance on overall survival. Median lenalidomide treatment arm was extrapolated to be 115 months, based on median of the control arms and HR (median, 86 months, HR = 0.75).}\

**McCarthy et al, JCO, 2017; 35:3273-3289\]**
Methods

MM patients enrolled in the RV MM COOP 0556 (EMN02/HO95 MM; NCT01208766)

- Newly diagnosed ≤ 65 years
- MRD assessment in patients achieving suspected CR before lenalidomide maintenance

Results

MRD status at pre-maintenance

Sub-analysis on MRD positive patients at pre-maintenance who had a second MRD evaluation > 1 year of Lenalidomide

76% Pre maintenance

LEN maintenance

Results

Progression free Survival: Median Follow-Up from MRD enrollment of 33 Months

3-yr PFS 77%

52%

N = 316

MRD negative

MRD positive

FISH – HIGH RISK

85%

ISS III

85%
73 NDMM patients on RV-MM-EMN-441, NCT01091831 (CRD vs Mel 200) and RV-MM-COOP-0556 NCT01208766 (VCD followed by VMP vs Mel 200) Both studies: len maintenance ASCT and no ASCT patients

Cancer 2018 in press
K-M estimates of PFS during Maintenance, PFS by Allelic-specific oligonucleotide real-time quantitative polymerase chain reaction (ASO-RQ-PCR) and Multiparameter flow cytometry (MFC)

m-CR, molecular complete response; flow-CR, flow-complete response; 73 patients started len maintenance.

ASO-RQ-PCR, median PFS for m-CR not reached vs 26 months for no-m-CR respectively p<0.001
MFC median PFS for MRD-negative not reached vs 19.5 months for MRD-positive respectively p<0.001

Gambella M et al Cancer 2018 in press
PFS during maintenance according to therapy (ASCT vs no ASCT) by ASO-RQ-PCR and MFC and ISS I vs ISS II/III
PFS during Maintenance according to Cytogenetic Risk

A/B: PFS Standard Risk Cytogenetics by ASO-RQ-PCR and MFC

C/D: PFS, High Risk Cytogenetics by ASO-RQ-PCR and MFC
Cavo M et al Blood 2012; Tacchetti et al EHA 2018

Einsele H et al Leukemia 2017

Sonneveld et al JCO 2012

Rosinol L et al Leukemia 2017

Mellqvist et al Blood 2013
Jackson et al ASH 2017

Transplant eligible pathway
Lenalidomide improved PFS from 30 to 57 months, hazard ratio of 0.47

Cytogenetic risk groups
Lenalidomide improved PFS irrespective of cytogenetic risk

- High risk: presence of any one of t(4;14), t(14;16), t(14;20), del(17p), or gain(1q).
- Ultra-high risk: presence of more than one lesion.
- Standard risk: absence of any of the above lesions.

Transplant eligible pathway
Lenalidomide improved 3 yr OS from 80.2% to 87.5%, hazard ratio of 0.69
Myeloma XI: MRD Testing by Flow Cytometry

30% of MRD + converted to MRD – with len compared to 4% on no maintenance ($p=0.0045$).
For MRD +: median plasma cells 0.13% on maintenance vs 0.39% $p=0.04$

MRD testing at start of and 6 months after maintenance
de Tute et al, ASH 2017; Blood 2017 130:90
BMT CTN 0702 Stem Cell Transplantation for Multiple Myeloma
Incorporating Novel Agents: SCHEMA

**Primary Endpoint: Progression-free Survival**

- **N=750 pts (250 in each arm)**
  - Register and Randomize
  - MEL 200mg/m²
  - VRD x 4*
  - Lenalidomide Maintenance**
  - MEL 200mg/m²
  - Lenalidomide Maintenance**

* *Bortezomib 1.3mg/m²
days 1, 4, 8, 11
Lenalidomide 15mg days 1-15
Dexamethasone 40mg
days 1, 8, 15
Every 21 days

**Lenalidomide x 3 years:
10mg/d for 3 cycles, then 15 mg/d
Amendment in 2014 changed Lenalidomide maintenance until disease progression after report of CALGB 100104.

Overall Survival

- **38 Month Estimate and 95% CI**
  - Auto/Auto: 82.0 (76.3, 86.5)
  - Auto/RVD: 85.7 (80.5, 89.5)
  - Auto/Maint: 83.4 (77.9, 87.7)

BMT CTN 0702: Regimens prior to Transplant

<table>
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<th>Initial Therapy</th>
<th>Auto/Auto (N=247)</th>
<th>Auto/RVD (N=254)</th>
<th>Auto/Maint (N=257)</th>
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<tr>
<td><strong>N</strong></td>
<td><strong>%</strong></td>
<td><strong>N</strong></td>
<td><strong>%</strong></td>
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<tr>
<td>Bort/Len/Dex</td>
<td>141</td>
<td>134</td>
<td>143</td>
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<tr>
<td>Cy/Bort/Dex</td>
<td>133</td>
<td>135</td>
<td>40</td>
</tr>
<tr>
<td>Len/Dex</td>
<td>24</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Bort/Dex</td>
<td>28</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Other</td>
<td>21</td>
<td>25</td>
<td>20</td>
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Bort, bortezomib; Cy, cyclophosphamide; Dex, dexamethasone; Len, lenalidomide
PFS by MRD status post induction

Kaplan-Meier plot for pfs by mrd6gp

Survival Probability

Survival Probability

Survival Probability

Survival Probability

PFS by MRD status pre-maintenance

Log-rank p-value: 0.002

Log-rank p-value: 0.002

Log-rank p-value: < 0.001

Log-rank p-value: < 0.001

PFS/OS by MRD status at one year on maintenance therapy

T Hahn, M Pasquini, P McCarthy, P Wallace

BMT CTN 0702
Analysis of focal lesions by multi-region sequencing

Spatial genomic heterogeneity in multiple myeloma revealed by multi-region sequencing

Spatial heterogeneity in myeloma

Multiple resistant sub-clones but MRD negativity...

Baseline 1. Relapses

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<th>Clone(s)</th>
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<td>KRAS Gly12Val</td>
<td>+144</td>
<td>sCR</td>
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<table>
<thead>
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<th>Clone(s)</th>
<th>5</th>
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<tr>
<td>KRAS Gly12Asp</td>
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04/2013 08/2013 11/2013 03/2014

VDTPACE + VRD

## Comparison of the Three Techniques

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<th>ASO PCR</th>
<th>NGF</th>
<th>NGS</th>
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<tr>
<td>% Informative Samples</td>
<td>Up to 70%</td>
<td>~100%</td>
<td>85-90%</td>
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<tr>
<td>Diagnostic Bone Marrow (BM) Sample</td>
<td>Needed for patient specific probes</td>
<td>Not needed</td>
<td>Needed or sample with enough myeloma cells</td>
</tr>
<tr>
<td>Plasma cells needed</td>
<td>Up to $10^6$</td>
<td>$5 \times 10^6$ or more</td>
<td>Up to $10^6$ (more if possible)</td>
</tr>
<tr>
<td>Fresh or processed sample</td>
<td>Either</td>
<td>Fresh</td>
<td>Either</td>
</tr>
<tr>
<td>Sample quality control</td>
<td>Cannot evaluate BM</td>
<td>Yes, analyze BM</td>
<td>Cannot evaluate BM</td>
</tr>
<tr>
<td>Standardization</td>
<td>Yes</td>
<td>Yes</td>
<td>Early</td>
</tr>
<tr>
<td>Availability</td>
<td>Yes in certified lab</td>
<td>Yes in certified lab</td>
<td>Two companies but not certified for clinical use</td>
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<tr>
<td>Sensitivity</td>
<td>$10^{-4}$ to $10^{-6}$</td>
<td>$10^{-5}$ to $10^{-6}$</td>
<td>$10^{-6}$</td>
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<tr>
<td></td>
<td>0.0001% to 0.000001%</td>
<td>0.00001% to 0.000001%</td>
<td>0.000001%</td>
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BMT CTN 1302: Study Outline

- Primary end point: PFS as a time to event from randomization
- Sample size: 138 patients (110 randomized patients)
BMT CTN 1302: Accrual to date (n=51, Rand, N=38) – 63% predicted

Projected
Actual
Projected Adj

Accrual Hold
March 2016
Chimeric Antigen Receptor (CAR) T cell therapy

• Gene transfer technology stably expresses CARs on T cells\(^1,2\)

• CAR T cell therapy takes advantage of the cytotoxic potential of T cells, killing tumor cells in an *antigen-dependent* manner\(^1,3,4\)

• Persistent CAR T cells consist of both effector (cytotoxic) and central memory T cells\(^3,4\)

• First human trial in resistant CLL patients\(^4\)

• **T cells are non-cross resistant to chemotherapy**


Original Slide Courtesy of D Porter
Bluebird BCMA CAR T Cells

- bb2121 is a second-generation CAR construct targeting BCMA, consisting of autologous T cells transduced with a lentiviral vector encoding a novel CAR incorporating an anti-BCMA scFv, a 4-1BB costimulatory motif to promote proliferation and persistence, and a CD3-zeta T cell activation domain.

- Construct demonstrated potent preclinical in vivo activity with low tonic signaling and showed BCMA-specific cell killing.

3 + 3 Dose Escalation of CAR + T Cells

<table>
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<tr>
<th>Dose Level</th>
<th>Quantity</th>
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<tr>
<td>1st Level</td>
<td>$50 \times 10^6$</td>
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<td>2nd Level</td>
<td>$150 \times 10^6$</td>
</tr>
<tr>
<td>3rd Level</td>
<td>$450 \times 10^6$</td>
</tr>
<tr>
<td>4th Level</td>
<td>$800 \times 10^6$</td>
</tr>
<tr>
<td>5th Level</td>
<td>$1200 \times 10^6$</td>
</tr>
</tbody>
</table>

*1200 $\times 10^6$ dose cohort no longer planned

Leukapheresis

Screening

Flu 30 mg/m²
Cy 300 mg/m²

Days -5, -4, -3

Day 0

BM BX (Wk 2)

BM BX (Wk 4)

1st Response Assessment (Wk 4)

Sample collections for T cell expansion & cytokines

bb2121 manufacturing
Manufacturing (10 days) + release

bb2121 infusion

Berdeja JG et al ASCO 2017
BCMA+ CAR T therapy For Multiple Myeloma

Fan et al. LBA3001 ASCO 2017
• 100% ORR
• 33/35 patients in remission within 2 months after BCMA CAR T therapy

Berdeja et al ASH 2017 Abs 740
• 85% ORR

November 17th, 2017
FDA Breakthrough Designation

Conclusions

• MRD Testing in MM patients after primary therapy
  – MRD can be tested by ASO PCR, NGF (MFC) and NGS
    • When to test?
      – After induction, before maintenance, at fixed time point after maintenance?
  – The level of sensitivity is important
    • Dependent on technique, quality of sample, % of malignant plasma cells and non malignant cells and total number of cells analyzed
  – MRD negativity after primary therapy appears to predict for outcome
  – Not all MRD negative patients remain in remission
  – Some MRD positive patients do not have disease progression
  – As therapies improve, early endpoints are critical for predicting long term outcome
  – There is a need to incorporate other factors such as immune profiling, cytogenetic stratification, PET-CT and Whole Body MRI to determine long term prognosis
  – Reference:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5664006/?report=classic
Carthage was the sworn enemy of Rome.

Cato the Elder ended all his speeches regardless of topic to the Roman Senate by saying, *Carthago delenda est, Carthage must be destroyed*, emphasizing the point of defeating Carthage.

In an era when median PFS are approaching >5 years and OS approaching 10 years, “Early surrogate endpoints for long term outcome (PFS/OS) must be tested in clinical trials so as to prevent studies that must remain open for 10 years or longer especially for an OS endpoint”
People and Services who make the BMT program possible

- S Balderman
- G Chen
- C Ho
- M Ross
- M Aungst
- M Burgess
- M Everett
- S Griebner
- A Koeppel
- J Lex-Sikinoff
- A Nemmer
- S Myszka
- A Phillips-Hall
- P Paplham
- R Russell
- A Beck
- S Flavin
- D Oliansky
- F Zhang
- A Kariapper
- H Jacobson
- R McKenzie
- S Oakley
- D Manfredi
- L Martin
- T Hahn
- M Herr
- S Schinnagel
- L Privitere
- K West
- J Pleskow
- M Clino
- M Steward
- D Swinnich
- K Stawicki
- D Cipolla
- K Dubel
- P Lipka
- S Siconolfi
- C Warren
- L Yoerg
- S Pry
- R De Wald
- L Markel
- R Kumpf
- K Dunn
- A Kader
- J Nichols
- H Bashaw
- S Clarke
- K Odunsi
- M Oprychal
- T Chodon
- R Koya
- C Choi
- A Hutson
- J Becker
- E Duman
- L Vesneske
- Rad Onc Service
- Radiology Svc
- Surgery Svc
- Pathology Svc
- Lab Medicine
- Stem Cell Lab
- Apheresis Unit
- S Szeglowski
- L Regan
- S Segal
- J Kapinos
- A Singh
- ID service
- B Segal
- N Almyroudis
- D DePaolo

- Managed Care and Finance Svc
- S Randolph
- M Budd
- Medical Oncology Fellows
- Leukemia, Lymphoma and Myeloma Services
- 5 East, 5 North and 6 North Nursing and Secretarial Staff
- Hospitalist Staff
- J Hillengass
- F Hernandez
- E Wang
- M Ernstoff
- J Lau
- E Repasky and Lab
- H Mohammadpour
- J Sarow
- P Wallace
- J Tario
- Y Zhang
- S Johnson
- C Johnson
- V Filadora
- B Segal
- T Schwaab
- A Kariapper
- N Almyroudis
- D DePaolo

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• The clinicians who provided care for these patients
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• The NCI: R Little, H Streicher
• RPCI: T Hahn, P Wallace, S Balderman, G Chen, F Hernandez, C Ho, K Lee, M Ross, P Torka, J Hillengass, P Wallace, J Tario
• Jane and our family who support my work schedule
Excitement on the horizon!

Lenticular Cloud over Chile which is reminiscent of a Red Blood Cell

Thank you very much!

Courtesy of Rosie McCarthy who found this on: http://www.flickr.com/photos/dcml/217552761/
Thank you very much
The Villain Returns: AML and ALL Relapse Following HCT and Treatment Strategies

Veronika Bachanova, MD, PhD
University of Minnesota

November 9, 2018
Outline

✓ Discover the incidence and continuing challenges of ALL and AML relapse following HCT
✓ Compare factors influencing detection of MRD in ALL and AML
✓ Describe the examples of novel therapeutic strategies to treat and prevent relapse in HCT patients
Relapse after HCT is the most common reason for transplant failure.

Survival after HLA-Matched Sibling Donor HCT for ALL, Age ≥18 Years, 2005-2015

A. Relapse and NRM for Adults, CR1/CR2

B. DFS and OS for Adults, CRS/CR2

Yessurun, Bachanova, Blood Advances 2018
Acute Myeloid Leukemia Overall Survival and Relapse

Survival after HLA-Matched Sibling Donor HCT for AML, 2005-2015

SOURCE: CIBMTR®, the research program of NMDP/Be The Match

Ustun C et al Leukemia 2017
Measurable Residual Disease is the Major Predictor for Relapse in Acute Leukemia

Methods of MRD monitoring in ALL

- **Multiparameter Flow Cytometry: 6-8 color immunophenotype** has sensitivity to 0.01% (1 out of 10,000 cells)
- PCR for IgH re-arrangement
- detection of BCR-ABL transcript by PCR with a sensitivity of 1/10,000
- FISH or cytogenetics (MLL gene re-arrangement, other)
- Bone Marrow (Standard) vs Peripheral Blood (not standard)
MRD after induction is the most critical high risk prognostic factor

Overall Survival of GMAAL Ph- patients, stratified by MRD after induction/early consolidation.

- B Overall Survival according to post-induction MRD.

1: Hematology 2017: 13-21
Impact of MRD Pre-HCT on Survival

- n=81
- Pediatric
- All Cell Type
- CR1-3
- IgH PCR

- n=56
- Pediatric
- B Cell
- CR1 and 2
- NSG IgVH vs. FC

- n=91
- Pediatric
- B Cell
- CR2
- IgVH PCR

**OSOS**
- n=81
- Pediatric
- All Cell Type
- CR1-3
- IgH PCR

**EFS**
- n=91
- Pediatric
- B Cell
- CR2
- IgVH PCR

Sutton et al. Bri J Haem 2014, the ANZCHOG ALL8 trial
Pulsipher et al. Blood 2015
Bader et al. JCO 2009 ALL Relapse Berlin-Frankfurt-Munster (ALL-REZ BFM) Study Group
MRD Impact Pre-HCT In CR1 Ph+ ALL in TKI era

- n=65
- Adult
- BCR/ABL1

Post-HCT Preventive Measures

OS

Lussana et al BBMT 2016
Impact of Conditioning (RIC vs MA) on Outcomes in Ph+ ALL with and without MRD

- **Registry (CIBMTR) analysis** of alloHCT for Ph+ ALL in CR1 using myeloablative and reduced intensity conditioning

  - 197 patients with Ph+ in CR1 (MAC 130 patients; RIC 67)
    - Matched pair (2:1) analysis

  - 70% received TKI pre-transplant

- Depth of remission was analyzed pre-HCT by FISH and/or RT-PCR
  - MRD negative 49% (MAC) and 39% (RIC)
Depth of remission (MRD) pre-HCT has significant impact on relapse.

Lowest relapse occurred in patients treated with TKI and MRD neg prior to HCT: 17% (MAC) and 20% (RIC).

Myeloablative alloHCT may overcome persistent minimal residual disease.

Bachanova V. et.al; Leukemia, 2014
Mar;28(3):658-65
Myeloablative and RIC yield similar survival for Ph+ ALL. A CIBMTR study

<table>
<thead>
<tr>
<th></th>
<th>RIC (n=67)</th>
<th>MAC (130)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS @3y</td>
<td>26%</td>
<td>28%</td>
<td>0.75</td>
</tr>
<tr>
<td>OS @3y</td>
<td>39%</td>
<td>35%</td>
<td>0.62</td>
</tr>
</tbody>
</table>

RIC is a valid alternative strategy for Ph+ ALL patients ineligible for MAC and MRDneg status is preferred pre-HCT

Overall Survival ~ 55% (TKI and MRDneg)

Bachanova V et al., Leukemia, 2014
Mar;28(3):658-65
Randomized comparison of prophylactic and MRD-triggered imatinib after alloHCT for Ph+ ALL

Imatinib 400-600mg/d

n=54 patients in CR1/CR2

Duration of administration **207** vs **121** days
2/3rds stopped imatinib prematurely

Pfeifer H et al., Leukemia 2013
Leukemia. 2013 Jun;27(6):1254-62
Randomized comparison of prophylactic and MRD-triggered imatinib after alloHCT for Ph+ ALL

DFS (5y) 83 vs 77%
OS (5y) 69 vs 62%

2 caveats:
Early molecular recurrence and/or transcripts >10*4 derived limited benefit from imatinib

Imatinib maintenance can reduce post alloHCT relapse

Pfeifer, H; Leukemia. 2013 Jun;27(6):1254-62
Immunotherapy Targets on B-cells

- Surface proteins targeted by immunotherapy
  - Rituximab
  - Ofatumumab
  - Obinotuzumab
Novel Immune based approach for cancer cell killing

- Bispecific T cell engager Blinatumomab
- CD22 Immunotoxin Inotuzumab
Blinatumomab is effective in ALL with MRD
Blinatumomab induced MRD- State Pre-HCT is Beneficial

<table>
<thead>
<tr>
<th>CR/CRi</th>
<th>MRD (n = 26) vs no MRD (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cox HR (95% CI)</td>
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<tr>
<td></td>
<td>Log-rank p:</td>
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<tr>
<td></td>
<td>MRD Response</td>
</tr>
<tr>
<td>MRD</td>
<td></td>
</tr>
<tr>
<td>Response</td>
<td></td>
</tr>
<tr>
<td>Median time from HSCT to OS (95% CI), Months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NE (11.1, NE)</td>
</tr>
</tbody>
</table>

Courtesy of Dr. Jabbour, Tandem Meeting 2018
Active post-HCT Relapse Prevention Clinical Trials

• Blinatumumab post –HCT
• Inotuzumab post-HCT
• Infusion of g/d T cell post HCT
• CAR-T19 for post HCT relapse?
Measurable Residual Disease in AML

• Multiparameter Flow Cytometry
• Multigene Next Generation Sequencing (targeted gene panels)
Somatic mutations associated with acute myeloid leukemia.

482 patients with AML

NGS

Average 13 mutations in each cell:
- DNA signaling genes (59%)
- Methylation-related genes (44%)
- Chromatin-modifying genes (30%)
- Myeloid transcription-factor genes (22%)
- Transcription-factor fusions (18%)
- Tumor suppressors (16%),
- Spliceosome-complex genes (14%)
- Cohesin-complex genes (13%).
AML....Complicated

- MANY RELATED CLONES
- DIFFERENT SIZES
- DIFFERENT GROWTH RATES

Courtesy of Dr. Radich
Initiating Mutations (\textit{DNMT3A, TET2, IDH1/2} are Less Likely to be Cleared than Cooperating Mutations (\textit{FLT3, NPM1, KRAS/NRAS})

Red=mutations that are not cleared on day 30

Klco et al, JAMA, 2015
DNMT3A, TET2, and ASXL1 had no effect

...but clinical implications of persistent mutations are very different

DNMT3A, TET2, and ASXL1 had effect

C Overall Survival among All Patients

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>Training cohort</th>
<th>Validation cohort</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Detection</td>
<td>No detection</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>164</td>
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<tr>
<td>No detection</td>
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<td>Detection</td>
<td>12</td>
<td>39</td>
</tr>
<tr>
<td>No detection</td>
<td>7</td>
<td>29</td>
</tr>
</tbody>
</table>

Jongen-Lavrencic NEJM 2018

Klco et al, JAMA, 2015
Negative Impact of MRD (FC) Pre-HCT

Daisuke Araki et al. JCO 2016

Ustun C et al. BMT 2013
The effect of MRD is important in patients with intermediate cytogenetic risk patients. It alters the prognosis of patients with FLT3+ patients, but it has no effect in adverse risk patients.

**THM**
- The effect of MRD is important in patients with intermediate cytogenetic risk patients.
- Alters the prognosis of patients with FLT3+ patients.
- But no effect in adverse risk patients.
Effect of MRD is important, but not constant in all AML Spectrum

- Complex Cytogenetics
- >CR2
- FLT3/Intermediate Risk
- Favorable Group
If MRD+ before HCT in AML, what can we do about it?

- Administer more chemotherapy (consolidations)
- Prevention of relapse after HCT
No Impact of Additional Postremission therapy on Sibling alloHCT for AML in CR1


Myeloablative Conditioning

OS

Relapse

RIC

Overall Survival (OS)

Cumulative Incidence of Relapse

Warlick E, BBMT 20, Issue 2, 2014, 202 - 208
Posttransplant Sorafenib to prevent relapse in high risk patients

Sorafenib maintenance in FLT3 + AML Patients

Brunner et al. BBMT, 2014(20):2042-8

Brunner et al. ASH 2015

Brunner et al Haematologica 2016
Other post HCT relapse prevention strategies in AML

• FLt3 Inhibitors
• Demethylating agents Azacytidine and Decitabine
• Tyrosine kinase Inhibitors (IDH2 etc)
• Immune-based approaches
Harnessing Natural Killer Cells for Cancer Therapy in AML

- Strategies to enhance donor NK cell function
- Bi and Tri-specific AML targeting NK cell engagers (BiKE, TriKE)

Natural killer (NK) cells play a critical role in infection control, tumor surveillance and cancer cell killing.
Donor derived NK Cells Are Alloreactive

Autologous

Normal Cell

Inhibitory Receptor

Activating Receptor

Inhibitory Ligand

Donor derived NK Cells Are Alloreactive

Allogeneic with KIR-HLA mismatch

Donor NK Cell

Cancer Target

Lysis
What Turns NK Cells On?

**Antibody-Dependent Cellular Cytotoxicity (ADCC)**
- IFN-γ
- TNF
- CD16
- Ab-coated cell
- Cytotoxic Granules

**Natural Cytotoxicity**
- IFN-γ
- TNF
- NK cell
- Infected or Tumor
- Cytotoxic Granules
IL-15 Super Agonist ALT-803 to Prevent Relapse Of High Risk Acute Myelogenous Leukemia and Myelodysplastic syndrome Following Allogeneic Stem Cell Transplantation

Participating Affiliate Institutions:
- Emory University
- Ohio State University
- University of Washington, Seattle
- Washington University at St Louis
A multicenter, open label Phase II Study of 6 mcg/kg sq once a week begin ALT-803 between Day 60 and Day 100 post-transplant

Primary Objective:
• CI of Relapse rate at 1 year after alloHCT

Eligibility:
Patients with high risk AML or with high risk MDS
FUTURE CLASS OF IMMUNOTHERAPY: Bi- Tri-specific NK Cell Engagers

BiKEs

BiKE-Mediated Killing

CD16
CD33

NK Cell

Immunological Synapse

Redirected Lysis

Tumor Cell

AML

TriKEs

Leukemia Cell

Anti-CD19
Anti-CD22

ALL

BiKE- Mediated Killing

Anti-CD16 Antibody

Anti-CD33 Antibody

CD16xCD33 BiKE

NK Cell

CD16

Leukemia Cell

Anti-CD19 Anti-CD22

ALL
Rationale For Trike

- T-CAR are successful because they are antigen specific and have a 41BB-L or CD28 intracellular domain to induce proliferation.
161533 TriKE enhance serial killing of AML Blasts

Legend:

Blue = NK cell
Green = Live AML
Red = Dead AML

AML is the HL60 Target

Bjorn Onfelt
Microbiology, Tumor and Cell Biology, Karolinska Institutet
IL15-CD33 Targeting TriKE: Preclinical Data and Phase 1 Trial

Phase 1 Study of CD16/IL-15/CD33 Tri-Specific Killer Engagers (TriKEs) for High Risk Heme Malignancies is enrolling patients
Questions?

Thank you!