The “AlloHSCT” and prospects for cellular therapy

Ghulam J Mufti

King’s College Hospital NHS Foundation Trust
Disclosures

• Research funding and advisory board of Celgene, Cellectis, Novartis
Presentation 7: Recent Advances...

5. Case Study - Mr X

- Previously fit, 65 year old man with symptoms of anaemia of 6 month duration.
- Bone marrow- hypercellular
- WCC- 1.6x10^9/l (N=0.3x10^9/l); Hb- 10.1G/dl; Plts- 21x10^9/l; Bone marrow blasts- 7%; cytogenetics- 47,XY,+8; Mutation-NRAS(G12A), HCT CI- 1; VUD donor 10/10; CMV -/-

Treatment Option

a. Demethylating agents alone
b. Demethylating agents followed by alloHSCT
c. Intensive chemotherapy followed by alloHSCT
d. AlloHSCT with myeloablative conditioning upfront
e. Reduced Intensity Conditioning with post-transplant MRD monitoring
f. a, b & c
Current treatments for MDS fail to cure MDS & only prolong survival by a few months at best

Therefore who is eligible?

Who will benefit?

How can benefits of HSCT best be increased in the context of leukaemia specific immunity
Relapse is the leading cause of treatment failure post HSCT

Prevention of relapse post HSCT now the most important challenge in AML/MDS
We have an ageing population

![The number of over-65s for every 100 adults](chart)

**World Population Prospects: the 2017 Revision**
Number of older people >60 years — In 2017 = 962 million; 2050 = 2.1 billion; 2100 = 3.1 billion.

**Patients who are eligible for HSCT are increasing due to a fitter population**
HSCT Activity in Europe 1990-2016:
Main indications/donor origin: allogeneic 1st. HSCT

2010 – 2014 activity (12,538)
>70yrs = 1,049 (8%)
65-70yrs = 4,026 (32%)
60-64yrs = 7,463 (60%)
How best to select for transplant?

1. Disease Subtype & IPSSR
2. Cytogenetic & Molecular
3. HCT-CI PAM
4. Disease status @ Transplant
5. Donor Characteristics
6. Combinations of 1-5
Kaplan-Meier analysis of OS & CIR following allogeneic HSCT in MDS patients stratified on their pre-transplant IPSS or IPSS-R risk

N=519
(2000-2011)

Matteo G. Della Porta et al. Blood 2014
Post-transplant outcome in MDS patients stratified according to the absence or presence of monosomal karyotype

A Overall survival

B Cumulative incidence of relapse

Matteo G. Della Porta et al. Blood 2014
Retrospective EBMT study of 579 patients confirms validity of IPSS-R at HSCT irrespective of prior therapy

Overall Survival

Median OS (months)
Very low: 23.6; Low: 55; Int: 19.7; High: 13.5; Very high: 7.8

Incidence of NRM

Multivariate Analysis Significant Factors
IPSS-R, graft source, age and prior treatment

Scheid BMT 2017
Overall survival according to remission status and percentage of marrow blasts

- Treated in CR1 (126 pts)
- RA-RARS, untreated (104 pts)
- RAEB-RAEBt-CMML, untreated (163 pts)
- Treated not in CR1 (130 pts)

Onida et al. Haematologica 2014
Intensive chemotherapy (ICT) +/- 5-Azacytidine (5-Aza)
Cytoreduction pre Allo-HSCT

Retrospective study of 265 patients, 163 of whom received cytoreductive therapy prior to transplant.
ICT=98, Aza= 48, ICT + AZA = 70, SIB = 75, VUD = 88, MAC = 33, RIC = 130

Damaj et al JCO 2012
Venetoclax combined with decitabine or azacitidine in treatment-naïve, elderly patients with acute myeloid leukemia

- 145 patients > 65yrs with treatment naïve AML
- Median age 75
- Poor risk cytogenetics in 49%
- Venetoclax (400-1200mg) and azacytidine 75mg/m² for 7 days OR Decitabine 20mg/m² for 5 days
- Median time on study 8.9 months

- CR + Cri for venetoclax 400mg + HMA cohort was 67%
- Median duration CR+Cri 11.3 months
- Median OS 17.5 months
- Median OS not reached in venetoclax 400mg group

Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia

Venetoclax + LD AraC
N=71
CR/CRI Intd Cyto = 76%, Poor Risk = 47%
Responses in all molec subtypes = 60-100%, except Tp53 mut = 44%

Pollyea et al, Nov 2018

DiNardo et al Blood October 2018
CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia

Lancet JE, Journal of Clinical Oncology. 2018
Post-Hoc Analysis suggests possible improved outcomes in those who received HSCT

A greater proportion of patients receive HSCT with CPX compared with 7+3²

34% CPX (52/153) 25% 7+3 (39/156)

Lancet JE, Journal of Clinical Oncology. 2018
TP53 and Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndromes

- Of the 116 patients, 53 (46%) achieved <5% BM blasts.
- Response rates were higher in patients with an unfavourable karyotype than an intermediate-risk or favourable-risk cytogenetic profile (29 of 43 [67%] vs. 24 of 71 [34%], P<0.001)
- Patients with TP53 mutations had a higher response rate than among patients with wild-type TP53 (21 of 21 [100%] vs. 32 of 78 [41%], P<0.001).

Phase I Trial of Allo-UCART123 in High Risk AML

UCART123 attributes:
- CD123 scFv (Klon43, murine)
- 2nd Generation CAR: CD3ζ + 4-1BB
- RQR8+: CD20 epitope to trigger cell destruction by anti-CD20 antibodies
- TRAC KO: to prevent GvHD

<table>
<thead>
<tr>
<th>Cohort number</th>
<th>UCART123 Dose 1</th>
<th>UCART123 Dose 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>2.5x10⁵/kg</td>
<td>2.5x10⁵/kg</td>
</tr>
<tr>
<td>1</td>
<td>6.25x10⁶/kg</td>
<td>6.25x10⁶/kg</td>
</tr>
<tr>
<td>2</td>
<td>5.05x10⁶/kg</td>
<td>5.05x10⁶/kg</td>
</tr>
</tbody>
</table>

Cytoreduction
Induction Chemotherapy (DA or FLAG-Ida)

Lymphodeplete FC UCAR123 1st Administration
28 days later → re-assess
28-32 days later 2nd UCART123

MRD by Immunophenotyping (0.1%)

2nd Cytoreduction DA Or FLAG-Ida
Blasts >20%

OFF Study

Blasts >20%

2nd UCART123 2nd Administration
28-32 days later

HSCT
Frequency of Driver Mutations and Age-Independent Association of TP53 Mutations with Poor Overall Survival

45 patients with truncating TP53 mutations (16%) had shorter survival than with missense mutations (P=0.005)

Models for Overall Survival, Including Clinical and Genetic Variables and Effect of Conditioning Intensity

- N=1514
  - (pMDS = 1203, tMDS = 311)
  - <5% blasts = 646
  - MAC = 789
  - RIC = 582
  - NMA = 130

MAC vs RIC – No difference

≥40yr of Age
  - RAS pathway Mutation
    - 129 Patients (9%)
    - 3yr OS, 30%
    - Median OS, 0.9yr
  - No RAS pathway Mutation
  - MAC better than RIC

≤40yr of Age
  - High-risk features
    - Therapy-related MDS
    - Platelets, <30x10⁹/litre at transplantation
    - Bone marrow blasts, ≥15% at diagnosis
  - ≥1 high-risk feature
    - 98 Patients (6%)
    - 3yr OS, 49%
    - Median OS, 2.6yr
  - No high-risk feature
    - 116 Patients (8%)
    - 3yr OS, 82%
    - Median OS, not reached

Effects of *TP53*, *RAS* and CK on survival and relapse

Tetsuichi Yoshizato et al. Blood 2017
Weighted contributions of clinical & genomic factors predicting survival because of leukemic relapse after BMT for MDS and sAML

Clinical factors (70%)
- Age ≥ 41.5 years
- RBC transfusion history (+)
- Diagnosis at HCT: sAML
- Disease status: relapsed/refractory
- Female to male donor
- Hematopoietic cell transplant-comorbidity index (HCT-CI) ≥ 3
- Performance status ≥ 3
- Days from diagnosis to HCT < 1275
- Disease status: Not CR

Genetic factors (30%)
- High-risk cytogenetic abnormality
- Complex karyotype
- TP53mut
- NRASmut
- CBLmut

Overall survival

Noa G. Holtzman, and Aaron P. Rapoport Blood 2017
Choosing an older sibling versus a Volunteer Unrelated Donor?

Effect on Allo-SCT Outcome

Rates of GVHD

Immune senescence

Stem Cell Exhaustion

Conflicting Data

EBMT data: improved survival in older MDS patient with use of young URD compared to MSD (Kroger et al 2012). NOT shown in other studies.

Early studies suggested a higher rate of GVHD with aged donors but this has not been consistent and conflicting data has emerged (Kolman et al 2001; Alousi et al 2013)

Impaired B cell, T Cell and NK compartments; decreased diversity; chronic inflammation

Stem cell Reserve decreases with ageing. Quality and Regenerative capacity of HSC obtained under GCSF mobilisation from an elderly donor is reduced
Role of donor clonal haematopoeisis in allogenic HSCT

1. 500 healthy, related HSCT donors (≥55yrs) targeted 66-gene panel sequencing
   - (1993 – 2017) Myeloid disease: 19.2 vs 6.3 (p<0.001)

2. 92 clonal mutations, median VAF of 5.9% in 80 (16.0%) donors
   - DNMT3a = 8%
   - TET2 = 2.2% (C→T)
   - ASXL1 = 1.4%

3. Alive patients median follow-up 3.3yr (0.1 - 20.6)
   - Higher cumulative incidence of cGVHD; hazard ratio (P=0.003)
   - Lower CIR/P (Univariate P =0.027; multivariate P=0.042)
   - No effect on non-relapse mortality and OS
   - 2 donor leukaemia's & lineage expansion of CHIP clone paralleled the fall in chimerism

CIBMTR (10,000 unrelated donor stem cell transplantation)
- 1999 – 2014 - younger donors are associated with a better transplant outcome
  Shaw et al Biol Blood Marrow Transplant. 2018

"Allogeneic HSCT from donors with CHIP seems safe and results in similar survival in the setting of older, related donors"
  Frick et al JCO 2018
Outcomes after cord blood transplant are limited by relatively high NRM

Gerds et al BBMT 2017

CIBMTR analysis: n=176
Median age 56 (18-73) years
OS = 31% at 3yrs
NRM = 40% at 3yrs
Relapse – 32% at 3 yrs

Robin et al BBMT 2018

N=833

Outcome after cord blood transplant are limited by relatively high NRM

Gerds et al BBMT 2017
Dose-Reduced Versus Standard Conditioning Followed by Allogeneic Stem-Cell Transplantation for Patients With Myelodysplastic Syndrome: A Prospective Randomized Phase III Study of the EBMT (RICMAC Trial).

129 patients with MDS and sAML

- Median age = 50
- Matched related (MAC/RIC) = 17/16
- Matched unrelated (MAC/RIC) = 36/38
- MM R/UR (MAC/RIC) = 11/11

- Randomly assigned 1:1
- OS at 2yrs = 76% (RIC), 63% (MAC)
- RFS at 2yrs = 62% (RIC), 58% (MAC)
- No difference in acute or chronic GVHD

**RIC (n=65)**
- Fludarabine: 150mg/m²
- Busulphan: 8mg/kg PO or 6.4mg/kg IV

**MAC (n=64)**
- Busulphan: 16mg/kg PO or 12.8mg/kg IV
- Cyclophosphamide: 120mg/kg

Kroger et al JCO 2017
Anti-lymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease

Randomised Phase-3 study patients assigned in a 1:1 ratio to receive ATG or not, with stratification according to center and risk of disease

- n=168, median follow-up = 24 months
- Chronic GVHD = 32.2% (with ATG) vs 68.7% (no ATG) (p<0.001)
- Chronic GVHD free and Relapse Free Survival at 2yrs = 36.6 (with ATG) vs 16.8 (no ATG) (p=0.005)
- Relapse free (p=0.21)/Overall survival (p=0.46) /Non-relapse mortality (p=0.6)
**Kings College Conditioning Regimen: Flu/Bu/Campath RIC Protocol**

**Cohort Details**

- Number=192 (86 F/ 106 M)
- Median Age =56 years (21-72)
- IPSS Low/ Int 1: 50 (26%)
  - Int 2/ High: 44 (23%)
- MDS/AML: 86 (45%)
- URD= 148 (77%)
- MMUD=41 (21%) -9/10

**Cohort Details – Survival at 5yrs**

- OS = 42%
- DFS = 33%
- RA/RCMD OS = 63%
- MDS-AML OS = 46%
- CMML/RAEB OS = 25% and 24%

**5-Year DFS**

- HCTCI 0-1 = 42%
- HCTCI >/= 2 = 24%
- p=0.01

*Potter et al BBMT 2014*
Mutation Clearance after Transplantation for Myelodysplastic Syndrome

- 86 of the 90 patients studies had a mutation
- Multivariate analysis showed that patients with a mutation and a variant allele frequency of at least 0.5% detected at day 30 had a higher risk of progression (P<0.001) and a lower 1-year rate of progression-free survival (P=0.002)
Down-regulation of MHC class II genes (HLA-DPA1, HLA-DPB1, HLA-DQB1, and HLA-DRB1) at relapse post transplant to levels 3 to 12 times lower than in paired samples obtained at presentation.

In haploidentical transplants at relapse loss of mismatched haplotype leads to the failure of the GVL effect. The remaining minor antigen alloreactions and NK cytotoxicity are insufficient to prevent leukemic proliferation.

Escape from GVL effect and selection of increasingly genomic unstable clones with multiple mutations in stem/progenitor cells
Post-Transplant/Relapse Strategies

Donor Lymphocyte Infusion
- Pre-emptive +/- DLI
- Therapeutic +/- DLI
- +/- Azacitidine +/- DLI
- Chemotherapy +/- DLI

Vaccination Strategies
- B7.1/IL2; WT1; etc

Check Point Inhibitors Therapy
- CTLA4; PD1; PDL1; etc

Neoantigens/Leukemia Associated Antigen Specific T cells

CAR123 Cellular Therapy

NK Mediated Cellular Therapy

Second Transplant

Antibody-based therapeutics (DARTs, BiTEs)
- CD33/CD3; MCLA-117/CD3; CD123/CD3
Different strategies for delivering DLI

Orti et al 2017
Early Administration of Pre-emptive DLI correlates with durable AML/MDS Remission

- Myeloid Malignancies
  - AML/MDS/MPN

64 patients

pDLI given if

- Donor CD3 <50%
- Falling donor CD3 >20% in one month

Escalating Dose DLI

- 6-8 Week Intervals

77% of patients achieved FDC or stable MDC after DLI
- 5yr OS was 91% in these patients

OS at 5 years after pre-emptive DLI – 80%
EFS at 5 years after pre-emptive DLI – 65%
GVHD incidence – 31% (19% chronic extensive) after pre-emptive DLI
Limitations to current data

- Above data → promising results but
  - Retrospective
  - Median time to DLI administration 6 months
  - Varying DLI schedule
  - Varying DLI dose
  - DLI given for mixed CD3 chimerism

- No randomised prospective data for DLI in MDS/AML
- No accompanying prospective translational data
- No evidence that pre-emptive or prophylactic DLI influences outcome in MDS/AML despite this being adopted as standard practice by many institutions
But what is the sweet spot?

![Graph showing the relationship between T cell dose of DLI (cells/kg) and timing of DLI following HSCT (months) with different risk levels: Low (<25% risk of GvHD), Medium (25%-50% risk of GvHD), and High (>50% risk of GvHD). The graph includes categories for No TCD, In vivo TCD, and Ex vivo TCD.]
Maintenance with 5-Azacytidine for Acute Myeloid Leukemia and Myelodysplastic Syndrome Patients

Prospective randomized controlled trial. Most patients did not complete the planned 12 cycles. Study closed early due to slow accrual

Oran et al ASH Abstract 971 Dec 3 5.30pm
Novel therapeutics following Relapse: AZA (=/- DLI)

Cancer/testis antigens are upregulated in vitro and in vivo by these drugs hypothetically sensitizing (“priming”) malignant cells to DLIs.

Schroeder et al 2015 BBMT: AZA and DLI for relapse

- 154 patients (AML n=24; MDS n=28)
- All patients received a median number of 4 courses of Aza (range, 4 to 14)
- DLI: 105 patients (68%; median DLI, 2; range, 1 to 7).
- CR and PR: 27% and 6%, respectively, resulting in an ORR of 33%.
- MVA identified molecular only relapse and MDS (as predictors for CR)
- OS at 2 years was 29% ± 4%

(Goodyear et al., Blood 2010, Almstedt et al., Leuk. Res. 2010),
DNMTi Induce Interferon Responses

- DNMTis induce an interferon response in cancer cells by activating dsRNA sensors
- DNMTis induce ERV demethylation and expression helping trigger the dsRNA response
- DNMTi viral defense genes in melanoma track with patient response to immune therapy
- DNMTi treatment sensitizes to anti-CTLA-4 immunotherapy in a melanoma mouse model
Ipilimumab for Patients with Relapse after Allogeneic Transplantation

- N=28 (Phase 1/1B)
- AML /MDS = 18
- CR = 5/18 (27%)
- Immune related toxicity = 21%
- GVHD = 14%
- Patients who had a complete response or stable disease had fewer CD4+ Treg cells and more CD4+ Tcon cells than patients with progressive disease.

# Impact of Neoantigens on survival in MDS

## Characteristics

<table>
<thead>
<tr>
<th>Patients with neoantigens</th>
<th>Patients with no neoantigens</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>129</td>
<td>51</td>
</tr>
<tr>
<td>Age in years (median)</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Sex (Male / Female)</td>
<td>86 /43</td>
<td>31 /20</td>
</tr>
<tr>
<td>Type of MDS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>RARS</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>RCUD</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>RCMD</td>
<td>42</td>
<td>10</td>
</tr>
<tr>
<td>Isolated 5q-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>RAEB</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>Other</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>IPSS Categories</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Low</td>
<td>46</td>
<td>15</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>55</td>
<td>20</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>High</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Number of mutations (median)</td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Progression to AML</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Yes</td>
<td>26/129 (20.1%)</td>
<td>8/51 (15.7%)</td>
</tr>
<tr>
<td>Vital Status</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Dead</td>
<td>28/129</td>
<td>15/51</td>
</tr>
</tbody>
</table>

Collaboration with Dresden

Tom Coats, et al 2018
Intracellular cytokine staining of CD8+ DILs after re-stimulation with mature DCs alone or WT1 mRNA-electroporated DCs

- N=30 High Risk AML
- Anti-leukemic response in 13
- 9/30: sustained CR
- 4/30: Stabilisation of disease
- Polyepitope WT1 specific CD8 response correlated with OS

Sébastien et al Blood 2017
Clinical courses of TCR-WT-11-E04 and TCR-WT-06-E03 after the WT-specific TCR-T cell transfer.

- HLA-A 24:02 restricted WT1 specific TCR redirected T-cells for AML-MDS
- Mutated WT1 peptide with adjuvant
- 16% CD8+ WT1 specific T cells
CD80/IL-2 immune gene therapy trials at Kings

- B7.1 is an immune co-stimulatory molecule and IL-2 a cytokine which stimulates an immune response.

- The “AML Cell Vaccine” (ACV) is patient specific, derived from the patients’ own AML cells which are modified ex-vivo to express B7.1 (CD80) and IL-2

- AML cells then g-irradiated and re-administered to the patient as a whole cell vaccine.

- Modification of the AML cells to express B7.1 and IL-2 enhances the efficiency of antigen presentation to the immune system, generating an anti-leukaemia immune response
CD80/IL-2 Immune Gene Therapy

Pre-study entry conditions:
- Relapsed AML/MDS, following allogeneic HSCT
- >50% donor CD3 chimerism at relapse
- <5% BM blast following cytoreductive chemotherapy
Delayed Type Hypersensitivity (DTH) following DLI + CD80/IL-2 AML cell vaccination

Patient T003 - 24h after 2\textsuperscript{nd} dose vaccination

Patient T003 - 24h after 3\textsuperscript{rd} and final dose vaccination

7 Patients enrolled to date:  - Confirmed feasibility  
- No acute toxicities / adverse events  
- Safety/efficacy studies completely satisfactory
Personalising the transplant for MDS
Optimising opportunities for success

Allogeneic SCT for Patients Age ≥ 70 Years with MDS: A Retrospective Study of the MDS Subcommittee of the Chronic Malignancies Working Party of the EBMT

N=313 Median age 72 (70-78)
MDS = 221, Sec AML = 92
MAC = 52, RIC = 207, Non myeloablative = 54
NRM at 1yr and 3y = 32% and 38%
OS = 34%

Decreasing disease burden pre-transplant
Tailor the treatment according to genetic and MRD profile
Recognise opportunities for excellent long-term outcomes in lower risk cases
Early adaptive immunotherapy and/or post-tx maintenance in high risk cases

Need for prospective randomized trials
5. Case Study - Mr X

- Previously fit, 65 year old man with symptoms of anaemia of 6 month duration.
- Bone marrow- hypercellular
- WCC- 1.6x10^9/l (N=0.3x10^9/l); Hb- 10.1G/dl; Plts- 21x10^9/l; Bone marrow blasts- 7%; cytogenetics- 47,XY,+8; Mutation-NRAS(G12A), HCT CI- 1; VUD donor 10/10; CMV -/-

**Treatment Option**

a. Demethylating agents alone  
b. Demethylating agents followed by alloHSCT  
c. Intensive chemotherapy followed by alloHSCT  
d. AlloHSCT with myeloablative conditioning upfront  
e. Reduced Intensity Conditioning with post-transplant MRD monitoring  
f. a, b & c
Acknowledgment

Kings Health Partners Bone Marrow Transplant Team

Dr Victoria Potter
Director of Bone Marrow Transplantation and Immune Effector Cell Therapy Program