Indicazione alla terapia immunosoppressiva nelle sindromi mielodisplastiche

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Two crucial observations

• some MDS (hypoplastic MDS, 10-15%) share clinical features with AA and more generally to BM failure disorders
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• some MDS (hMDS, 10-15%) share clinical features with AA and more generally to BM failure disorders

• Immunosoppressive therapy has been reported to be effective in a significant percentage of patients with MDS
AA vs MDS

- Easily distinguished when marrow hyper- or normocellular, obvious dysmorphic changes and cytogenetic abnormalities, increase percentage of blasts

- In about 15% MDS, marrow hypocellular, morphology subtle and insufficient metaphases

- Evolution of some AA to MDS with abnormal cytogenetics and response of some MDS to immunosuppression

Is hMDS a distinct entity?

- Patients tend to be younger (< 60y)
- Have profound neutropenia and thrombocytopenia
- Have a lower percentage of blasts
- They less likely display abnormal karyotype
- They usually have a more favourable course
- They usually respond to IST
MDS and T cell immune disregulation

- High levels of TNFα and IFNγ have been reported\(^1,2\)
- Expansions of T cell clones with limited TCR-V\(\beta\) repertoire\(^3\)
- WT1 protein (overexpressed in MDS with trisomy 8 abnormality)\(^4\)
- Presence of PNH clones\(^5\)

Trisomy 8 MDS

Direct CD8+ antigen specific proliferative response to trisomy 8 antigen (WT1)

T cell mediated suppression of healthy and abnormal bone marrow progenitors

Sloand EM et al Blood 2005; 106:841
Molecular model of T cell pathogenesis in MDS

- CD8+
- CD4+
- CD8+
- CD8+
- IL2Rγ common cytokines: IL7, IL2, IL15, IL-21
- Homeostatic proliferation of multiple self antigens
- Break in peripheral tolerance due to expansion of self-reactive CD8 T cells
- Increase of Th17
- Depletion of Treg
- Apoptosis of HPCs and cytopenia

Zou JX et al. Leukemia 2009; 23(7):1288-96
Detection and significance of clonal populations with a paroxysmal nocturnal hemoglobinuria (PNH) phenotype

PNH clones are reliably detected in many patients with aplastic anemia and MDS, although the clone size is generally small (<5%) and the significance remains controversial.
PNH

• PIG-A gene on X chromosome

• Single somatic mutation sufficient

• Deficiency of GPI membrane-anchored proteins

• Manifestations:
  – Complement-mediated hemolysis
  – Thrombosis
  – Aplastic anemia
  – Rarely leukemia
Diagnostic Test for PNH

- Flow Cytometry performed on peripheral blood
- Quantitative results expressing clone size on both granulocytes and erythrocytes
  - Erythrocytes alone are not sufficient due to hemolysis and the dilution effect of transfusions
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD55, or FLAER

Padova approach: panel for cytometric analysis in patients with suspected PNH
The bacterial toxin Aerolysin

1. Proaerolysin is a 52-kDa protein secreted by *Aeromonas hydrophila*. After proteolytic nicking at the C-terminus, the active form, Aerolysin, binds to cell surface structures and oligomerizes, forming channels that result in cell lysis.

2. Aerolysin does not lyse PNH cells

3. It was used to enrich rare GPI-negative PNH clones.

4. An Alexa 488-linked version of a non-lysing, mutated form of proaerolysin (FLAER) was generated with specificity for GPI-linked structures but unable to cause cell lysis.

5. FLAER cannot be used to assess PNH clones in the erythrocyte lineage, since the latter do not possess surface-bound proteolytic enzymes needed to process the Proaerolysin.

*D.R. Sutherland, 2007*
Comparison between FLAER and CD66b
Bone Marrow Failure

PNH Haemopoiesis

Normal Haemopoiesis

Normal
• The most widely accepted mechanism for clonal expansion of PNH-type cells in patients with BM failure is the “escape hypothesis” which states that the relative number of PIG-A mutant HSCs increases by avoiding immunological attacks by T cells.

• It should be further noted that the finding of such clones has not been related to clinical hemolysis, and specific PNH therapy is not indicated.
PNH Clone in MDS

• GPI-anchored proteins: CD16, CD66b, CD55, CD59

• PNH cells (>1%) in 22% of 115 patients with AA and 23% of 39 with MDS; correlated with response to immunosuppression
  • Dunn DE, Ann Intern Med, 1999

• High incidence of an expanded PNH clone (>1%) in 136 patients with AA (32%) and MDS (18%); stable proportion over time
  • Maciejewski JP, Br J Haematol, 2001
PNH Clone

• Minor population (>0.003%, range 0.003% to 2.41%) in 17.6% of RA, but none in other MDS

• Less than 1% in 17 of 21 patients

• associated with:
  – less karyotypic abnormality (4.8% vs 32.8%)
  – less progression to AML (0% vs 6.2%)
  – Higher response to CSA (77.8% vs 0%)
  – Higher incidence of HLA-DR15 (90.5% vs 18.5%)

Wang H, Blood, 2002
HLA Alleles

- HLA-DR15 (DR2) in 36% of 72 MDS and 42% of 59 AA

- Higher than controls (blood donors, 21.3%); p=0.01 for MDS and p<0.001 for AA

- In IBMTR, 30% of MDS and 33% of AA patients were HLA-DR2+

- In MDS, younger age and short duration of RCTD also predicted response
Immunosuppressive Agents used to treat MDS (IST)

- Anti-Thymocyte Globulin (ATG)
- Ciclosporin A
- Anti-TNF drug (Etanercept)
- Anti-CD52 (Campath)
ATG (anti –Thymocyte globulin)

ATG is a mixture of purified polyclonal IgG from the sera of horses (hATG; 15/40 mg/kg/day x 4/5 days) immunized with human thymocyte (Lymphoglobulin and Atgam) or Jurkat cell line (ATG Fresenius) or rabbit (rATG, Thymoglobulin; 2.5/3.5 mg/kg/day x 5 days)
Mechanism of Action of ATG

- Broad antibody specificity; antibodies to T-cells, activated B-cells, NK-cells, and monocytes
- T cell depletion in PB and lymphoid tissues through complement-mediated lysis and apoptosis
- Modulation of key cell surface molecule
- Interference with dendritic cell functional properties
- In vitro colony stimulation
- Proliferation and differentiation of normal progenitors
**Relationship to Dose**

- Toxic to purified AA, MDS, and normal BM CD34+ cells at high conc (100 – 1000 $\mu$g/ml)

- Increased colony growth in BM CD34+ cells of AA and MDS patients at low concentration (1-10 $\mu$g/ml)

- rATG similar activity but 10-fold more potent than hATG

- Serum ATG during therapy: 100 – 1000 $\mu$g/ml; Serum ATG 1-2 months after: 0.1 - 10 $\mu$g/ml

Response to hATG in MDS

- 61 pts with MDS, at least bi-lineage dysplasia, transfusion-dependent and off steroids/CSA/growth factors
- Median age 60, 61% RA, 16% RARS, 23% RAEB
- Rx: ATG 40mg/kg daily x 4, prednisone
- 34% became transfusion-independent

Molldrem JJ, Ann Intern Med, 2002
Factors Predicting Response

• Univariate
  • Favorable
    – Younger patients
    – MDS subtype RA
    – Lower platelet count
  • Unfavorable
    – Abnormal karyotype
    – Anemia as the only cytopenia
    – Hypercellular marrow
    – Older age
    – Higher platelet count

• Multivariate
  – Age p=0.005
  – Platelet count p=0.038
  – Marrow cellularity p=0.10

Molldrem JJ, Ann Intern Med, 2002
Experience with Thymoglobulin

- German multicenter study:
  - 35 pts with MDS; 24 RA, 10 RAEB, 1 CMML
  - Median age 63 (range 41 -75 years)
  - Randomized to hATG (Lymphoglobulin) 15 mg/kg x 5 days (n=20) or Thymoglobulin 3.75 mg/kg x 5 days (n=15)
  - Response in 12 of 35 pts (34%), 4 CR
  - Time to response 1 – 10 months (median 3)
  - Response duration 1 – 17+ months (median 9)
  - Both safely administered and no difference in RR

Stadler M, Leukemia, 2004
<table>
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<th>Author (pub year)</th>
<th>Country</th>
<th>N</th>
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Calcineurin inhibitors (CNIs)

• Ciclosporin A (CyA) (Neoral®)
  – binds to intracellular protein, cyclophilin
    • only active once bound

• Tacrolimus (FK506) (Prograf® & Advagraf®)
  – binds to intracellular protein, FKBP-12
    • only active once bound
Mechanism of action of CyA and Tacrolimus
Ciclosporin A

- **Oral absorption**
  - V large inter & intra-patient variability
  - IV dose & administration
- **Distribution** – extensive (not dialysed)
- **Metabolism**
  - CYP450 3A4 with adult half-life 5-18 hours
    - Shorter in children
    - Steady state 2-3 days after change of dose
### Clinical Trials of CyA based regimen

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Other IST in MDS

- anti TNF-α (Etanercept), associated to ATG
- anti-CD52 (Campath)  

1Deeg HJ, Leuk Res 2004; 2Sloand EM, JCO 2010
Long term outcome of IST-treated patients?

- Leukemia occurred exclusively in the non-responding patients older than 60y (int-1 or higher)

MDS that occurs in younger patients elicits a more powerful anti-myeloid lineage immune response, resulting in more significant cytopenia, whereas MDS developing in older patients is characterized by a more pronounced stem cell defect, leading to development of leukemia
3.4.1. Recommendations

Existing evidence indicates that the use of immunosuppressive therapy is appropriate for patients with MDS low-INT1 IPSS risk score who need a treatment, have <5% blasts in the bone marrow and do not have poor-risk cytogenetics (grade B).

The Panel agreed that the best candidates for immunosuppressive treatments are those with an age <60 years (grade B), a normal karyotype (grade B), a hypoplastic bone marrow (grade C) and the HLA-DRB1-15 antigen (grade C).

The use of ATG alone (grade C) or in combination with CysA (grade B) is recommended.
Thymoglobulin: Target Antigens

- T lymphocytes: CD3/TCR, CD2, CD4, CD5, CD6, CD8, CD27, CD28, CD96, CDw150, CD152...
- Integrins, MHC I...
- Granulocytes: Other shared epitopes
- Red blood cells
- Platelets

TCR = T-cell receptor; MHC = major histocompatibility complex
Basic mode of action

- (Drug + immunophilin) inhibits calcineurin
  - Prevents dephosphorylation (activation) of NF-Act T-cell
  - Factors which stimulate cytokine (i.e. IL-2/IFN-\(\gamma\)) gene transcription
  - Net result: impaired IL-2 production

- ‘reversible inhibition of T-cell activation, proliferation & clonal expansion’
  - Stops cell cycle at G0-G1 stage