Post transplant complications – VOD

An hypothesis on a unifying concept for the amplification of the endothelial lesion in VOD and aGvHD

A critical checkpoint and an innate immunity potential target.

Grant Prentice* and Gunther Eissner UCD BSBMT, London Oct. 15th 2014

*Conflicts – Jazz/Gentium consultant/prior Int. CMO
What is VOD/SOS?

- Occlusion/thrombosis of the liver sinusoids
- 1st described after consumption of Bush teas contaminated with alkaloids
- A “frequent” complication of HSCT/BMT 0–60+% of patients undergoing HSCT
- A life threatening disorder >80% mortality in severe cases

- Defibrotide is licensed in Europe for the treatment of sVOD and under FDA review

VOD = veno occlusive disease. SOS = sinusoidal obstructive syndrome

Sinusoids are small, irregular, vascular spaces which are closely surrounded by hepatocytes. Also found in the spleen and bone marrow and characterised by “fenestrations”
Clinical presentation of VOD

- VOD is characterised by rapid weight gain, ascites, heptomegaly, jaundice and right upper quadrant pain\(^1,2\)
- Symptoms usually present within first 2–4 weeks following SCT but can occur later\(^3\)
- VOD is a progressive disease:
  - Severe VOD is associated with MOF and a high mortality rate (>80%)\(^4\)

Diagnosis of VOD

• Liver biopsy is the gold standard for definitive diagnosis of VOD\(^1\)
  – However it is not standard practice and is considered an unacceptable risk

VOD is therefore diagnosed by clinical criteria
  – Based on the classical triad of weight gain, hepatomegaly (painful), and jaundice\(^2,3\)
  – Complex because the signs and symptoms of this condition often overlap with other processes\(^4\)

  ❖ Ultrasound is of limited value and can neither prove, nor disprove, the diagnosis

## Current clinical diagnostic criteria

<table>
<thead>
<tr>
<th>Seattle Criteria</th>
<th>Baltimore Criteria</th>
</tr>
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<tbody>
<tr>
<td><strong>To day +20 post-HSCT two of:</strong></td>
<td>Hyperbilirubinaemia &gt;2 mg/dL</td>
</tr>
<tr>
<td>• Bilirubin &gt;2 mg/dL (~34 µmol/L)</td>
<td>&lt;day +21 post-HSCT + two of:</td>
</tr>
<tr>
<td>• Hepatomegaly, RUQ pain</td>
<td>• Hepatomegaly</td>
</tr>
<tr>
<td>• Ascites +/- unexplained weight gain of &gt;2% baseline</td>
<td>• Ascites</td>
</tr>
<tr>
<td></td>
<td>• Weight gain &gt;5% from baseline</td>
</tr>
</tbody>
</table>

**Modified Seattle:** Similar to above with two differences:

1. Up to **day 30** post-HSCT
2. Ascites +/- unexplained weight gain of >5% baseline

NB both require a diagnoses of exclusion

Sev. VOD, + Renal or Pulmonary failure or encephalopathy or combinations
Understanding the pathophysiology and risk factors for VOD/SOS
Current understanding; the pathophysiology of Tx related VOD

**Conditioning regimen**  
Chemo/DXT

**Endothelial cell damage**  
Direct and cytokine mediated e.g. TNFa, IL1b, IL-6, etc.

**↑ INFLAMMATION** and release of TF/reduced TF inhibitor  
+ CAMS. Monocytes and neutrophils and APCs release further cytokines

**↑ COAGULATION** triggered by TF

**↓ FIBRINOLYSIS** by release of PAI-1* + ↓ tPA

**Fibrin deposition**  
Hepatic venous outflow obstruction  
VOD

PAI-1 = Plasminogen Activator Inhibitor -1
Space of Disse

Sinusoid

Hepatocyte

P-450 enzymatic system

Glutathione enzymatic system

Toxic metabolites (Acrolein)

Non-toxic metabolites

Busulfan
TBI
BCNU
Etoposide
Risk factors for post Tx VOD

- Pre-existing liver damage with elevated transaminases/viral hepatitis/iron overload
- Underlying disease; e.g. Osteopetrosis, Familial histiocytosis or Neuroblastoma, advanced disease.
- Type of transplant: allogeneic vs autologous and
  - A higher incidence with high intensity conditioning e.g. BU/CY deplete Glutathione or Mylotarg and direct endothelial toxicity predispose patients to VOD.

Plus - GvHD prophylaxis

- Oestrogen Rx (x10 fold) - Heparanase gene expression increased
Risk factors for VOD in children

- Incidence (%)
- n=176

- Soft Tissue Sarkoma
- ALL
- AML
- MDS
- Neuroblastoma
- MAS
- Osteopetrosis

+Wolman's Disease (lysosomal acid lipase deficiency)
VOD incidence/GVHD prophylaxis with novel agents (mTORi): Rapamycin(Sirolimus)+Tacrolimus and increased incidence of VOD (N=488)

<table>
<thead>
<tr>
<th></th>
<th>VOD incidence n (%)</th>
<th>Odds ratio to reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tac/Mtx</td>
<td>16 (7)</td>
<td></td>
</tr>
<tr>
<td>Tac/Sir/Mtx</td>
<td>28 (21)</td>
<td>3.23 (1.61–6.69, p&lt;0.001)</td>
</tr>
<tr>
<td>Tac/Sir</td>
<td>15 (11)</td>
<td>1.55 (0.69–3.48, p=0.33)</td>
</tr>
</tbody>
</table>

Tac/Mtx, tacrolimus/methotrexate; Tac/Sir/Mtx, tacrolimus/methotrexate/sirolimus; Tac/Sir, tacrolimus/sirolimus

Cutler C et al. Blood 2008;112:4425-4431
Epidemiology of VOD in Tx 2

• Recent data (CIBMTR unpublished) suggest a decline in the overall incidence of VOD to ~8%.

• The donor source: Auto ~1.2%. Allo BMT 8%, ~75% occur with unrelated donors (vs ~25% siblings)

• The risk is reduced with T cell depletion, suggesting an immune related component

• The incidence has fallen with the increased use of RIC in adults and ? DF prophylaxis in children.

• Treatment with DF has reduced the mortality rate in severe VOD from 78% to 25%²

VOD – the cytokine cascade
Endothelial injury/inflammation 1

• TNFa and IL-1b are directly toxic to ECs
• They induce leucocyte adhesion molecule expression - E-selectin (ELAM-1), VCAM-1 and ICAM-1 (ligand for LFA-1) on the endothelial cell surface
• Pts, monocytes and neutrophils adhere to the ECs signaling cytoskeletal actin re-organisation through Rho/Rac - forming gaps and capping of the adhesive molecules, allowing cellular egress - an active process.
VOD - the Cytokine/Coagulation cascade

2. Intravascular coagulation

- Tissue Factor is released from the inflamed ECs, activating the extrinsic clotting cascade
- \((\text{VIIa} \rightarrow \text{Xa} \rightarrow \text{prothrombin} \rightarrow \text{Thrombin etc})\)
- The fibrinolysis inhibitor, plasminogen activator inhibitor-1 (PAI-1) is synthesized by inflamed ECs and its release is stimulated by TNFα and IL1β.
- PAI-1 elevation and reduced tPA leads to hypo-fibrinolysis.
Endothelial cells are the primary target in both VOD and GvHD

- The vascular endothelium is the primary target in VOD, GvHD (Holler Team)\(^1\), engraftment syndrome, idiopathic (haemorrhagic) pneumonia syndrome (aka DAH), TAM and capillary leak

- The endothelium is composed of ECs and the extracellular matrix (ECM)

Early post transplant complications

-The prothrombotic and hypofibrinolytic sequelae seen in VOD are well understood, but -

? what is the trigger for the “cytokine storm” that amplifies the vascular endothelial damage initiated by the “conditioning” and cellular egress in VOD and GvHD?
The ECM – Heparan Sulphate proteoglycans (HSPG) and heparanase

• The proteoglycans are receptors, in-activators and storage ligands for cytokines, chemokines and growth factors at the ECM and the plasma membrane of cells.

• HSPG activates organisation of the cytoskeleton and cell migration. It has a highly conserved NFkappaB binding site essential for responsiveness to TLR agonists (e.g. LPS).

• Heparanase – breaks down heparan sulphate proteoglycans releasing their stored contents including cytokines that trigger thrombosis, cellular adhesion and cellular egress into the target tissues.
Mechanism of VOD – a possible common checkpoint with aGvHD - and endothelial injury

Polymorphisms of the heparanase gene are associated with a higher risk of severe aGvHD.

“Highly significant correlation of HPSE gene SNPs rs4693608 and rs4364254 and their combination with the risk of developing acute GvHD – where the donor genotype predicts for higher levels of heparanase mRNA”

Ostrovsky O et al. *Blood* 2010;115:2319–2328 (A Nagler team)
VOD/Acute GvHD cellular effectors

- Adhesive molecules target platelets, monocytes and neutrophils to the inflamed ECs releasing further cytokines
- VOD: gaps appear between the endothelial cells allowing egress of nts., monos. and rbc’s into the Space of Disse causing sinusoidal compression
- aGvHD: the effector cells, donor cytotoxic T lymphocytes, are activated by professional APCs and non professional APCs - Activated endothelial cells function as APCs presenting host histocompatibility +/- minor antigens
- Disruption of the ECM activates CAMs, including CD99, that enable T cell egress to the target tissues - active process in aGvHD
Amplification and the Innate immune system in VOD/GvHD - TLR9

- Receptor for bacterial CpG DNA
- TLR9 is expressed on APCs and ECs and triggers an innate immune response
- TLRs also activate the adaptive immune system via APCs - to produce cytokines, chemokines and to express co-stimulatory molecules
- Selective decontamination, retaining certain Lactobacilli, reduces the incidence and severity of aGvHD
DF receptor – TLR-9  Endothelial cells express TLR-9

Eissner et al., unpublished observation
DF and TLR-9 co-localize in HMEC-GFP transfectants

Echart, Eissner et al., unpublished observation
Also .... TLR-9 antagonist H154 blocks the anti-apoptotic effect of DF

Costello, Eissner et al., unpublished observation
Potential points for intervention in VOD

- Conditioning regimens
- Identification of genetic predisposition
- Ursodiol (Ruutu, Blood Sept 2002)
- Drug levels
- GSH
- N-acetylcysteine

Inflammation
- Pentoxyphyline (anti-TNF), TNF antibodies, corticosteroids

Risk

Cell injury

Microthrombosis
- PGE
- Heparin
- AT III
- APC
- LMWH
- tPA
- Defibrotid

Necrosis

Fibrosis

TIPS
Liver transplant
Charcoal hemofiltration
CVVHD

TNF, tumor necrosis factor; GSH, glutathione; PGE, prostaglandin E; AT III, antithrombin III; APC, activated protein C; LMWH, low molecular weight heparins; tPA, tissue plasminogen activator; TIPS, transjugular intrahepatic portosystemic shunt; CVVHD, continuous veno-venous hemodialysis
What is defibrotide?

• Defibrotide is:
  – A mixture of oligonucleotides obtained from porcine intestinal mucosa
  – Prepared by controlled depolymerisation of DNA
  – Available in vials containing 200 mg solution for IV administration

DNA = deoxyribonucleic acid. IV = intravenous
Proven MOA – defibrotide

- Defibrotide protects and stabilises hepatic vascular endothelial cells
- Increases tissue thromboplastin inhibitor and reduces TF release
- Decreases anti fibrinolytic PAI-1
- Activates tPA – thrombolysis
- Inhibits heparanase gene transcription reducing cytokine release, adhesive molecule expression and markers of endothelial cell damage eg vWF and the coagulation cascade

Inflammation: Adhesion Receptors +/- DEFIBROTIDE

**HUVEC**

**HMEC**

**HMEC+DF**

**VCAM**

Carreras team
Thrombogenicity: Expression of VWF/TF+/-DEFIBROTIDE

HMEC

TF

VWF

TF + DF

VWF + DF

Partículas de oro/µm²

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<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Day 0</th>
<th>+7</th>
<th>+14</th>
<th>+21</th>
</tr>
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<tbody>
<tr>
<td>TF</td>
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<tr>
<td>VWF</td>
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<td>TF</td>
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<tr>
<td>VWF</td>
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Conventional treatment of VOD

• Current standard treatments are supportive, including:
  – Fluid/electrolyte correction including diuretics, transfusion and analgesia

• Systemic anticoagulants +/- thrombolytics
  – associated with severe bleeding complications
  e.g. rtPA (thrombolytic)
  – Response in up to 30% of patients
  – But increased risk of bleeding - no benefit in OS

• Liver transplant if all else fails

tPA = tissue plasminogen activator
Treatment of VOD with corticosteroids - The Pros and cons -

Pros:
1. The cytokine storm is a major component in the aetiology of VOD and will be partially suppressed by corticosteroid treatment
2. Conflicting reports for efficacy +ve e.g. High dose methylprednisolone for the treatment of veno-occlusive disease of the liver in paediatric haematopoietic stem cell transplant recipients. Myers et al. BBMT. 19 (2013) 492-513 (retrospective review) n=15. Rx used 500mgs 12 hrly for 3 days then tailed. Some had defibrotide 2-5 days after start of steroids (reason unknown but 3 of 4 had very high Bilirubin). Survival 78%. All had severe VOD. No serious toxicities reported.

Cons:
1. No inhibitory effect on the thrombotic component
2. No pro-fibrinolytic up-regulation
3. Increased risk of infection, in particular invasive fungal infection
   Corticosteroids paralyse the phagocytic properties of Monocyte/macrophages and neutrophils (reversed in part by the use of G/GM-CSF)
4. -ve reports e.g. Hennenfent et al, BMT 2005:37:229 n = 20 +24 in FU). Same Steroid protocol. RR 21%. Failure was due to GvHD or relapse in responders and infection and organ failure in non responders (58%. ND)
Recent clinical trials in treatment and prevention of VOD + Speculation on potential benefits in GvHD.
Recent clinical studies of defibrotide in the prevention or treatment of VOD

<table>
<thead>
<tr>
<th>Study (ID)</th>
<th>Disease status</th>
<th>Patients (age)</th>
<th>Dose(s) DF 2 hour infusions</th>
<th>Country (centres)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomised open-label dose-finding study (99-118)</td>
<td>Severe VOD post-HSCT</td>
<td>149 (Adult and paediatric)</td>
<td>6.25 and 10 mg/kg IV/6 hrly</td>
<td>USA (6)</td>
<td>Richardson (2002) Blood</td>
</tr>
<tr>
<td>European paediatric prevention trial (Eudra-CT 2004-000592-33)</td>
<td>HSCT at risk of VOD</td>
<td>360 (Paediatric)</td>
<td>6.25 mg/kg IV/6 hrly</td>
<td>EU (28)</td>
<td>Corbacioglu (2010) Bone Marrow Transplant Lancet (2012)</td>
</tr>
<tr>
<td>Pivotal, historically controlled, study in patients with severe VOD (2005-01)</td>
<td>VOD post-HSCT</td>
<td>134 (Adult and paediatric)</td>
<td>6.25 mg/kg IV/6 hrly</td>
<td>USA (19)</td>
<td>Richardson (2009) Blood 114, Abstract 654</td>
</tr>
<tr>
<td>International compassionate use programme</td>
<td>VOD</td>
<td>1129 (Adult and paediatric)</td>
<td>10–80 mg/kg/d</td>
<td>EU, USA, Asia (331)</td>
<td></td>
</tr>
<tr>
<td>Treatment IND study: Interim analysis (2006-05)</td>
<td>VOD</td>
<td>183 (Adult and paediatric)</td>
<td>6.25 mg/kg IV/6 hrly</td>
<td>USA (1)</td>
<td>Richardson (2012) ASH</td>
</tr>
</tbody>
</table>
Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial

Prof Selim Corbacioglu MD a, Simone Cesaro MD b, Maura Faraci MD c, Dominique Valteau-Couanet MD d, Bernd Gruhn MD e, Attilio Rovelli MD f, Jaap J Boelens MD g, Annette Hewitt BSc h, Johanna Schrum MD i, Ansgar S Schulz MD i, Ingo Müller MD k, Jerry Stein MD l, Robert Wynn FRCPath m, Johann Greil MD n, Prof Karl-Walter Sykora MD o, Prof Susanne Matthes-Martin MD p, Prof Monika Führer MD q, Anne O'Meara FRCP q, Jacek Toporski MD r, Prof Petr Sedlacek MD s, Prof Paul G Schlegel MD t, Karoline Ehler MD u, Prof Anders Fasth MD v, Prof Jacek Winiarski MD w, Johan Arvidson MD x, Prof Christine Mauz-Körholz MD z, Prof Hulya Ozsahin MD aa, Andre Schrauder MD ab, Prof Peter Bader MD ac, Prof Joseph Massaro PhD ad, Prof Ralph D'Agostino PhD ad, Margaret Hoyle BSc ae, Massimo Iacobelli MD ae, Prof Klaus-Michael Debatin MD i, Prof Christina Peters MD p x, Prof Giorgio Dini MD e x
Study design

Eligible patients N=360

Excluded n=4

Randomisation

Conditioning

30 day post-HSCT

Prophylaxis arm
• 4 x Defibrotide 25 mg/kg/day IV
• Given on first day of conditioning until 30 post-HSCT

No VOD

Control arm
• No prophylaxis

VOD

Min14 days treatment

Continue treatment until resolution (or death)

Crossover

No VOD
DF significantly reduces the incidence of VOD in children

40% reduction in VOD

p = 0.0488
Defibrotide reduces the incidence of MOF

<table>
<thead>
<tr>
<th>MOF (in all ITT patients)</th>
<th>Defibrotide (n=180)</th>
<th>Control (n=176)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of MOF</td>
<td>14 (8%)</td>
<td>18 (10%)</td>
<td>ns</td>
</tr>
<tr>
<td>• Renal failure</td>
<td>2 (1%)</td>
<td>10 (6%)</td>
<td>0.017</td>
</tr>
<tr>
<td>• Respiratory failure</td>
<td>14 (8%)</td>
<td>16 (9%)</td>
<td>ns</td>
</tr>
<tr>
<td>• Encephalopathy</td>
<td>1 (1%)</td>
<td>3 (2%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

- Mortality was four times higher in patients with VOD than those without VOD at 100 days post-HSCT (25% versus 6%; p<0.0001)
- The composite score for morbidity and mortality was significantly lower in the defibrotide group (p=0.034)**

*p value from X² test
Early post transplant complications

- Studies by Richardson et al suggested a reduction in aGvHD with the Rx of VOD by DF
- Confirmed in CIBMTR database analysis (Gentium/Jazz data)
- Corbacioglu et al\textsuperscript{2} confirmed this observation in a prospective randomised study of prophylactic DF (paeds) and supported by a reduction in use of corticosteroids.

DF also reduced the incidence and severity of acute GvHD

Incidence of GvHD by Day 100 post-HSCT

* p value from X² test for incidence if GvHD by Day 100 post-SCT
** p value from Wilcoxon test for grading of GvHD by Day 100 post-SCT

Severity of GvHD by day 100 post-SCT

<table>
<thead>
<tr>
<th>Grade</th>
<th>DF (n=122)</th>
<th>Control (n=117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 (25%)</td>
<td>33 (28%)</td>
</tr>
<tr>
<td>2</td>
<td>18 (15%)</td>
<td>30 (26%)</td>
</tr>
<tr>
<td>3</td>
<td>5 (4%)</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>4</td>
<td>4 (3%)</td>
<td>4 (3%)</td>
</tr>
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</table>

Grade II-IV GvHD was significantly reduced (p=0.013). severity by grade of GvHD by Day 100 post-SCT was significantly reduced in the DF group (p=0.0034)**

Gentium data on file, clinical summary report Eudra-CT 2004-000592-33
DF significantly reduced the need for corticosteroids

Corticosteroids, prescribed predominantly for acute GvHD, were also used significantly less in patients receiving DF compared with controls (68% vs 82%; p=0.0363)
No loss of anti leukamic activity
- Relapse of malignant disease:

<table>
<thead>
<tr>
<th>Disease (most frequent)</th>
<th>Relapse by D+100</th>
<th>Relapse by D+180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF (n = 180)</td>
<td>Control (n = 176)</td>
</tr>
<tr>
<td>ALL</td>
<td>4% (1/26)</td>
<td>18% (4/22)</td>
</tr>
<tr>
<td>AML</td>
<td>10% (3/31)</td>
<td>5% (2/42)</td>
</tr>
<tr>
<td>Other leuks</td>
<td>13% (1/8)</td>
<td>0 (0/5)</td>
</tr>
<tr>
<td>MDS</td>
<td>0 (0/20)</td>
<td>0 (0/11)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>6% (2/34)</td>
<td>6% (2/33)</td>
</tr>
<tr>
<td>Total</td>
<td>4% (8/180)</td>
<td>6% (11/176)</td>
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</tbody>
</table>
2005-01 Pivotal study of defibrotide for treatment of severe VOD

- An open-label, phase III, historically-controlled trial conducted between July 2006 and November 2008 at 35 centres across the USA, Canada and Israel

**Study objective**

- To evaluate the **safety** and **efficacy** of defibrotide for the treatment of severe VOD (patients with VOD and MOF) following HSCT
Defibrotide increases complete response

Primary end point met

Improvement in complete response

- **Defibrotide**
  - 37% (16/44) \( p=0.036 \)
  - 24% (24/102)

- **Historical control**
  - 7% (1/14) \( p=0.0131 \)
  - 9% (3/32)

\*p value adjusted by quintiles of propensity score based on four stratification variables: (1) Allo/auto HSCT, (2) adult/paediatric, (3) 1 or 2+ HSCTs, (4) ventilator/dialysis dependent
Defibrotide reduces mortality rate (100 days post-HSCT)

Mortality rate end point

Decrease in mortality rate

\[ p = 0.0341 \]

*P value adjusted by quintiles of propensity score based on four stratification variables: (1) Allo/auto HSCT, (2) adult/paediatric, (3) 1 or 2+ HSCTs, (4) ventilator/dialysis dependent
Pathophysiology of VOD and GvHD - An algorithm for future management?

Gene polymorphisms - examples*

- IL1b
- IL-2
- TNFa
- IL6
- IL-10

Cytokine gene polymorphism profile to predict the balance of pro vs anti-inflammatory and the impact on the conditioning induced cytokine storm in GvHD and VOD

- GSH predicts toxic metabolites
- TLRs (?2,3,4,5,9) NOD2/CARD15 etc and the GI Microbiome?
- Heparanase polymorphisms already proven for aGvHD (Nagler group)

To include; conditioning regimen, underlying disease and stage, age, prior treatment, allo (HLA matched, mismatched, gender), source i.e. BM vs PBSC, and cord, T cell depletion, post transplant immune suppressants, viral status (e.g. CMV, hepatitis viruses), other liver disorders such as iron overload

- ? An algorithm for prophylaxis planning in the future?

*Dickinson et al, 2004 British Journal of Haematology, 127, 479-490
# Vascular endothelial toxicity management conclusions

1. **• Reduce risk eg avoid high risk drugs for vascular endothelial and GI toxicity and the microbiome**

2. **• Develop a genetic polymorphism algorithm to define risk eg TNFa, GSH, heparanase and TLRs/NOD2/CARD15**

3. **• Identify the risk and consider prophylaxis or -**

4. **• Identify markers predictive of early endothelial damage for pre-emptive Rx eg PAI-1, vWF, adhesion molecules, FDPs, cytokines, circulating CD34 +ve damaged endothelial cells?**
   
   **• Predictive aGvHD biomarkers?**
Thank you for listening