

SUPPLEMENTARY INFORMATION

IL-6 RESPONSIVENESS OF CD4⁺ AND CD8⁺ T CELLS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION DIFFERS BETWEEN PATIENTS AND IS ASSOCIATED WITH PREVIOUS ACUTE GRAFT-VERSUS-HOST DISEASE AND PRETRANSPLANT ANTITHYMOCYTE GLOBULIN THERAPY

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Supplementary Table S1. Intracellular signaling in CD3⁺ mononuclear cells; a comparison of patients with and without previous acute GVHD. The table shows the effects of four forms of IL-6 stimulation on the phosphorylation of the intracellular mediators Akt(Thr308), mTOR(Ser2448), STAT3(Ser727) and STAT3(Tyr705) in CD3⁺ circulating mononuclear cells. All p-values refer to a statistical comparison between cultures with and without IL-6 activation signals; the corresponding control cultures thus contained PMA alone or PMA+anti-CD3+anti-CD28. The table shows the combinations with increased mediator phosphorylation corresponding to p<0.05. All comparisons were done using the Wilcoxon Signed Rank Test.

Phosphotarget	H-IL-6	IL-6	IL-6 sIL-6R	IL-6 sIL-6R sgp130FC
All patients				
AKT(Thr308)				
mTOR(Ser2448)				0.02
STAT3(Ser727)				
STAT3(Tyr705)	0.005			0.04
Patients with acute GVHD				
AKT(Thr308)				
mTOR(Ser2448)				
STAT3(Ser727)				
STAT3(Tyr705)	0.02		0.04	
Patients without acute GVHD				
AKT(Thr308)				
mTOR(Ser2448)				
STAT3(Ser727)				
STAT3(Tyr705)				

Supplementary Table S2. The effects of IL-6 stimulation on circulating CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells derived from patients with and without ATG as a part of their pretransplant GVHD prophylaxis. The effects of four different forms of IL-6 stimulation on the phosphorylation of Akt(Thr308), mTOR(Ser2448), STAT3(Ser727) and STAT3(Tyr705) were investigated. All p-values refer to a statistical comparison between cultures with and without IL-6 activation signals; the corresponding control cultures thus contained medium alone or anti-CD3+anti-CD28. The table shows the combinations with increased mediator phosphorylation corresponding to p<0.05.

Phosphotarget	Without TCR ligation				With TCR ligation			
	H-IL-6	IL-6	IL-6 sIL-6R	IL-6 sIL-6R sgp130FC	H-IL-6	IL-6	IL6 sIL-6R	IL-6 sIL-6R sgp130
CD3⁺CD4⁺ T cells; patients treated with ATG								
AKT(Thr308)								
mTOR(Ser2448)								0.04
STAT3(Ser727)	0.01	0.04						
STAT3(Tyr705)	0.002	0.007	0.007	0.004	<0.001	<0.001	<0.001	0.02
CD3⁺CD4⁺ T cells; patients not treated with ATG								
AKT(Thr308)								
mTOR(Ser2448)								0.02
STAT3(Ser727)					0.01		0.01	
STAT3(Tyr705)	<0.001	0.005	0.001	0.003	<0.001	<0.001	<0.001	<0.001
CD3⁺CD8⁺ T cells; patients treated with ATG								
AKT(Thr308)						0.02		
mTOR(Ser2448)								
STAT3(Ser727)	0.01	0.02						
STAT3(Tyr705)	0.002				0.01			
CD3⁺CD8⁺ T cells, patients not treated with ATG								
AKT(Thr308)								
mTOR(Ser2448)							<0.001	0.003
STAT3(Ser727)					0.02	0.03		
STAT3(Tyr705)					<0.01			

Supplementary Table S3. The effects of PMA stimulation on T cell subsets. The table shows the effects of PMA alone and PMA+anti-CD3+anti-CD28 on the phosphorylation of STAT3/Akt/mTOR in circulating CD3⁺CD4⁺ T cells, CD3⁺CD8⁺ T cells and CD3⁻ mononuclear cells derived from allotransplant recipients treated with ATG or not treated with ATG. All p-values refer to a statistical comparison between cultures with and without PMA activation signals; the corresponding control cultures were prepared in medium alone. The table shows the stimulations with increased mediator phosphorylation corresponding to p<0.05. All comparisons were done using the Wilcoxon Signed Rank Test.

	CD3 ⁺ CD4 ⁺		CD3 ⁺ CD8 ⁺		CD3 ⁻
Phosphotarget	PMA alone	PMA anti-CD3 anti-CD28	PMA alone	PMA anti-CD3 anti-CD28	PMA alone
Patients treated with ATG					
AKT(Thr308)		0.01	0.002	0.001	0.04
mTOR(Ser2448)	0.002	0.01	<0.0001	0.01	<0.001
STAT3(Ser727)	0.01		0.001		0.005
STAT3(Tyr705)	0.004				
Patients not treated with ATG					
AKT(Thr308)	<0.001	0.001	0.002	<0.001	0.03
mTOR(Ser2448)	<0.001	<0.001	<0.001	<0.001	<0.001
STAT3(Ser727)	<0.001	0.002	0.005	0.002	0.02
STAT3(Tyr705)		.			

Supplementary Table S4. Effects of IL-6 stimulation on intracellular signaling in CD3⁺ mononuclear cells; a comparison of patients with and without previous ATG therapy. The effects of four forms of IL-6 stimulation on the phosphorylation of the intracellular mediators Akt(Thr308), mTOR(Ser2448), STAT3(Ser727) and STAT3(Tyr705) in CD3⁺ circulating mononuclear cells were investigated. All p-values refer to a statistical comparison between cultures with and without IL-6 activation signals; the corresponding control cultures thus contained medium alone or anti-CD3+anti-CD28. The table shows the combinations with increased mediator phosphorylation corresponding to p<0.05. All comparisons were done using the Wilcoxon Signed Rank Test.

Phosphotarget	H-IL-6	IL-6	IL-6 sIL-6R	IL-6 sIL-6R sgp130
Patients treated with ATG				
AKT(Thr308)				
mTOR(Ser2448)				0.03
STAT3(Ser727)				
STAT3(Y705)	0.007			
Patients not treated with ATG				
AKT(Thr308)				
mTOR(Ser2448)				
STAT3(Ser727)				
STAT3(Y705)				