

Supplemental Material for

Biomarker Panel for Chronic Graft-Versus-Host Disease

Jeffrey Yu, Barry E. Storer, Kushi Kushekhar, Mohammad Abu Zaid, Qing Zhang, Philip R. Gafken, Yuko Ogata, Paul J. Martin, Mary E. Flowers, John A. Hansen, Mukta Arora, Corey Cutler, Madan Jagasia, Joseph Pidala, Betty K. Hamilton, George L. Chen, Iskra Pusic, Stephanie J. Lee, and Sophie Paczesny

Supplemental Methods

Proteomics

The overall proteomics workflow is shown in Appendix Fig. A1 and detailed methods are presented below.

Enzyme-linked immunosorbent assay (ELISA)

Candidate protein validation was performed with a sequential ELISA protocol.¹⁻³ Commercial antibody pairs were available for 22 proteins (Table S1), and ST2 and CXCL9 ELISAs were performed as previously reported.^{4,5} Samples and standards were analyzed in duplicate as described previously.¹⁻³

Statistical analysis

Clinical differences in the groups with and without cGVHD were compared with Student's t tests for continuous variables and Fisher's exact tests for categorical variables. Logistic regression was used to evaluate the associations between cGVHD and biomarkers after log transformation. All analyses were adjusted for significant clinical variables considering age, sex, stem cell source, conditioning (myeloablative vs. others), donor (matched sibling vs. others), and time from HCT to sample collection, and for steroid use in verification cohort 2. To determine the best combination model, we used forward selection with a 0.05 significance threshold, confirmed by backward selection. Receiver operating characteristic (ROC) curves were generated and the corresponding areas under the curve (AUC) were calculated to determine the best

single biomarker and combination model. For day 100 samples in verification cohort 2, Cox regression was used to evaluate the association between biomarkers and subsequent incidence of cGVHD over the next 3, 6, and 12 months, including cases of cGVHD that arose among controls from the case-control analysis. Death and relapse were treated as competing risks. ROC curve analysis was performed, treating the small number of patients lost to follow-up or competing risks before the 6- and 12-month landmarks as non-cGVHD events. Differences in cGVHD severity between groups were evaluated using the Wilcoxon two-sample tests. The analysis of NRM divided the panel-weighted sum on the median value among cGVHD cases in both verification cohorts and compared cases above and below the median. NRM was estimated using cumulative incidence methods, treating relapse as a competing risk, and compared between groups using Cox regression. All statistical analysis was performed using SAS (Cary, NC).

Proteomics

Sample preparation and protein fractionation

Each pool contained 25 µl of plasma. The three pooled plasma samples were then individually immunodepleted of the 20 common hyper-abundant proteins with a ProteoPrep®20 plasma immunodepletion kit (Sigma-Aldrich) according to manufacturer's protocol. The flow-through fractions (depleted plasma) were then concentrated using Vivaspin® 500 (Vivaproducts). After measuring protein concentrations of depleted and concentrated plasma with a Micro BCA protein assay reagent kit (ThermoFisher Scientific), 50-µg aliquots were precipitated using acetone at

-20°C overnight. After centrifugation for 20 minutes at 15,000×g, acetone was decanted, and the air-dried protein pellets were dissolved in 25 µL of 1.0 M triethylammonium bicarbonate, 1 µL of 2% sodium dodecyl sulfate (SDS), and 2 µL of 50 mM tris-(2-carboxyethyl)phosphine (TCEPP) provided in an iTRAQ® reagents application kit ⁶ – plasma and incubated for 1 hour at 60°C. Cysteine residues were alkylated by adding 1 µL of 84 mM iodoacetamide in water to each vial and incubating for 30 min in darkness. All samples were trypsinized by adding 10 µg trypsin (Sigma-Aldrich) in 10 µL water and incubating them overnight at 37°C. Each pool was labeled with a different tag allowing for differential quantification. Three iTRAQ reagents,⁶ 114–116, were added in 70 µL ethanol to each vial along with 10 µL sample buffer plasma to adjust the pH to greater than 8, and the vials were incubated at room temperature for 1 hour. The samples were labeled in the following order: 1) de novo cGVHD with label 114, 2) control with label 115, and 3) progressive cGVHD with label 116. The labeling reaction was quenched by adding 100 µL water and incubating the tubes for 30 min at room temperature. All vials were dried in a speed vac separately and stored at -20°C until fractionation by strong cation-exchange (SCX) chromatography.

The three pooled plasma samples were dissolved in buffer A (7mM potassium phosphate, 30% acetonitrile, pH 2.65) and combined immediately before fractionation with a SCX column (Zorbax 300-SCX 5 µm, 2.1 × 150 mm, Agilent). The sample pH was adjusted to less than 3 with 2% trifluoroacetic acid (TFA). Fractions were collected at 1 minute intervals at a flow rate of 200 µL/min from 1% solvent B (7 mM potassium phosphate, 500 mM KCl, 30% acetonitrile, pH 2.65) to 60% over 40 min (1% B for 7 minutes, 6–15% B for 23 min, 15–34% B for 15 min, and 34–60% B for 10 min) as well

as during column washing with 98% solvent B for 10 min. The chromatographic elution was monitored using a UV detector at $\lambda=220$ nm. These fractions were consolidated into 20 fractions using the UV trace to distribute the peptide quantities similarly. After drying them in a speed vac, peptides were desalted using Sep-Pak® C18 (50 mg) cartridges. The cartridges were conditioned with 1 mL acetonitrile, 2 × 1 mL of 65% acetonitrile in 0.1% TFA in water followed by 2 × 1 mL of 0.1% TFA. After sample loading in 1 mL of 0.1% TFA and 2 washes with 1 mL of 0.1% TFA, peptides were eluted using 1 mL of 65% acetonitrile in 0.1% TFA in water and dried in a speed vac.

LC-MS/MS analysis

LC-MS/MS analysis was performed with an Easy-nLC 1000 (Thermo Scientific) coupled to an Orbitrap Elite mass spectrometer (Thermo Scientific). The LC system configured in a vented format⁷ consisted of a fused-silica nanospray needle (PicoTip™ emitter, 75 μm ID, New Objective) packed in-house with 25 cm of Magic C18 AQ 100Å reverse-phase media (Michrom Bioresources Inc.), and a trap (IntegraFrit™ Capillary, 100 μm ID, New Objective) containing 2 cm Magic C18 AQ 200Å. The peptide sample was diluted in 30 μL of 2% acetonitrile and 0.1% formic acid in water, and injection volumes ranging between 4-8 μL were loaded onto the column in triplicate and separated using a two-mobile-phase system consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). A 90-min gradient from 7% to 35% B at a flow rate of 400 nL/min was used for chromatographic separation. The mass spectrometer was operated in a data-dependent MS/MS mode over the m/z range of 400–1800. The precursor scan mass resolution was set to 60,000. For each cycle, the 10 most abundant ions from the precursor scan were selected for MS/MS analysis using 40% normalized HCD collision

energy and analyzed in the orbitrap with the resolution set to 15,000. Selected ions were dynamically excluded for 45 seconds.

Peptide and protein identification from mass spectra of digested fragments

The acquired LC-MS/MS data were analyzed using two approaches.⁸ In the first approach, Proteome Discoverer™ version 1.4 was used for data analysis. The MS/MS data were searched against the Swiss-Prot human proteome database (release 2012_01, 74127 entries) using SEQUEST⁹ with the following parameters: trypsin was set as the digestion protease, with 2 maximum missed cleavages; precursor and fragment error tolerance were 10 part per million (ppm) and 0.6 Dalton, respectively; iTRAQ modification of N-termini was a fixed modification; and iTRAQ modification of lysine residues, carbamidomethyl on cysteine residues, and oxidation of methionine residues as variable modifications. Identified peptides were filtered according to a 1% peptide-level false discovery rate (FDR) using Percolator.¹⁰ Proteins with at least one identified peptide were reported.

In the second approach, the mass spectra were searched against the same database using Mascot™ version 2.4 with the same parameters as used in the first approach. Identified proteins were then filtered with the significance threshold $p < 0.05$ and at least one identified peptide.

The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium¹¹ via the PRIDE partner repository with the dataset identifier PXD002762.

References

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Table S1: List of 105 proteins with differential ratio in the proteomics experiment

Gene name	prot_accession number	prot_description	114/115 protRatio	116/115 protRatio	Gene Ontology Process(es)	ELISA available
ACAN	P16112	Aggrecan core protein OS=Homo sapiens GN=ACAN PE=1 SV=2	0.37	0.52	extracellular matrix organization and disassembly, cell adhesion, keratan sulfate catabolic process, proteolysis	Yes
ADAMTS13	O76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13 OS=Homo sapiens GN=ADAMTS13 PE=1 SV=1	0.74	0.72	cell-matrix adhesion, integrin-mediated signaling pathway, proteolysis, response to interferon-gamma, tumor necrosis factor	
ADAMTSL4	F8WAD0	ADAMTS-like 4 OS=Homo sapiens GN=ADAMTSL4 PE=4 SV=1	0.72	0.86	cellular adhesion, angiogenesis	
ARHGDI3	P52566	Rho GDP-dissociation inhibitor 2 OS=Homo sapiens GN=ARHGDI3 PE=1 SV=3	1.57	1.30	Rho protein signal transduction, actin cytoskeleton organization, cellular response to redox state, immune response	
ATRN	O75882	Attractin OS=Homo sapiens GN=ATRN PE=1 SV=2	0.75	0.67	inflammatory response, myelination, response to oxidative stress	
AVIL	O75366-2	Isoform 2 of Advillin OS=Homo sapiens GN=AVIL	0.61	0.85	actin filament capping, cilium morphogenesis, cytoskeleton organization	
C1RL	Q9NZP8	Complement C1r subcomponent-like protein OS=Homo sapiens GN=C1RL PE=1 SV=2	0.74	0.80	complement activation, classical pathway, innate immune response, proteolysis	
CD44	P16070-3	Isoform 3 of CD44 antigen OS=Homo sapiens GN=CD44	0.89	0.75	cell-cell interactions, cell adhesion, cell	Yes
CD5L	O43866	CD5 antigen-like OS=Homo sapiens GN=CD5L PE=1 SV=1	0.80	0.51	apoptosis, cellular defense response, receptor-mediated endocytosis	
CDH5/VE cadherin	P33151	Cadherin-5 OS=Homo sapiens GN=CDH5 PE=1 SV=5	0.81	0.75	adherens junction organization, blood vessel maturation, cell junction assembly	Yes
CDHR2	Q9BYE9	Cadherin-related family member 2 OS=Homo sapiens GN=CDHR2	1.93	1.01	cell adhesion	
CLEC3B	P05452	Tetranectin OS=Homo sapiens GN=CLEC3B PE=1 SV=3	0.70	0.68	bone mineralization, cellular response to transforming growth factor beta stimulus	
COL1A1	P02452	Collagen alpha-1(I) chain OS=Homo sapiens GN=COL1A1 PE=1 SV=5	0.50	0.62	cartilage and bone development, cellular response to epidermal growth factor, cellular response to fibroblast growth factor, collagen fibril organization, collagen catabolic process	
COL11A2	C9J8W5	Collagen, type XI, alpha 2 OS=Homo sapiens GN=COL11A2 PE=4 SV=1	0.59	0.65	cartilage development, collagen fibril organization, collagen catabolic process	
COL15A1	P39059	Collagen alpha-1(XV) chain OS=Homo sapiens GN=COL15A1 PE=1 SV=2	0.75	0.78	cell adhesion, collagen catabolic process	
COMP	P49747	Cartilage oligomeric matrix protein OS=Homo sapiens GN=COMP PE=1 SV=2	0.75	0.82	extracellular matrix organization, limb development, cell adhesion, apoptosis	Yes
CPN1	P15169	Carboxypeptidase N catalytic chain OS=Homo sapiens GN=CPN1 PE=1 SV=1	0.81	0.70	proteolysis, response to glucocorticoid, bradykinin catabolic process	
CR2	P20023-3	Isoform C of Complement receptor type 2 OS=Homo sapiens GN=CR2	0.50	0.79	B cell differentiation, B cell proliferation, immune response, virus receptor activity	
CRTAC1	Q9NQ79-3	Isoform 3 of Cartilage acidic protein 1 OS=Homo sapiens GN=CRTAC1	0.68	0.91	extracellular matrix organization, negative regulation of receptor binding	
DPEP2	Q9H4A9	Dipeptidase 2 OS=Homo sapiens GN=DPEP2 PE=1 SV=2	0.82	0.66	proteolysis	
DPP4	P27487	Dipeptidyl peptidase 4 OS=Homo sapiens GN=DPP4 PE=1 SV=2	0.92	0.72	T cell activation, endothelial cell migration, negative regulation of extracellular matrix disassembly, proteolysis	
ECM1	Q16610	Extracellular matrix protein 1 OS=Homo sapiens GN=ECM1 PE=1 SV=2	0.71	0.86	angiogenesis, inflammatory response, bone mineralization, T cell migration	Yes
ENG/CD105	P17813	Endoglin OS=Homo sapiens GN=ENG PE=1 SV=2	0.73	0.77	cell adhesion, cell chemotaxis, bone morphogenetic protein signaling pathway	Yes
ENO1	P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	1.12	1.53	canonical glycolysis, response to virus	
FAP	Q12884	Seprase OS=Homo sapiens GN=FAP PE=1 SV=5	0.72	0.84	angiogenesis, cell adhesion, endothelial cell migration, proteolysis	Yes
FBN1	P35555	Fibrillin-1 OS=Homo sapiens GN=FBN1 PE=1 SV=3	0.73	1.00	cellular response to transforming growth factor beta stimulus, extracellular matrix disassembly, sequestering of bone morphogenetic protein in extracellular matrix	
FCN3	Q5SSB9	Ficolin (Collagen/fibrinogen domain containing) 3 (Hakata antigen) OS=Homo sapiens GN=FCN3 PE=2 SV=1	0.74	0.85	complement activation, lectin pathway, innate immune response	Yes
FGA	Q6NSD8	FGA protein OS=Homo sapiens GN=FGA PE=2 SV=1	0.89	2.29	acute phase response, adaptive immune response, blood coagulation, cell-matrix adhesion, extracellular matrix organization, fibrinolysis	
FGB	P02675	Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2	0.85	1.85	acute phase response, adaptive immune response, blood coagulation, cell-matrix adhesion, extracellular matrix organization, fibrinolysis	
FGG	P02679-2	Isoform Gamma-A of Fibrinogen gamma chain OS=Homo sapiens GN=FGG	0.86	2.27	acute phase response, adaptive immune response, blood coagulation, cell-matrix adhesion, extracellular matrix organization, fibrinolysis	

FN1	P02751	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=4	1.30	8.20	acute phase response, angiogenesis, blood coagulation, cell-matrix adhesion, extracellular matrix organization, leukocyte	Yes
GBP1	P32455	Interferon-induced guanylate-binding protein 1 OS=Homo sapiens	3.86	0.85	cytokine-mediated signaling pathway, defense response to virus, interferon-gamma-mediated signaling pathway	
HIST1H2BA	Q96A08	Histone H2B type 1-A OS=Homo sapiens GN=HIST1H2BA PE=1 SV=3	1.54	2.40	chromatin organization, inflammatory response, mononuclear cell migration,	
HIST1H2BB	P33778	Histone H2B type 1-B OS=Homo sapiens GN=HIST1H2BB PE=1 SV=2	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BC	P62807	Histone H2B type 1-C/E/F/G/I OS=Homo sapiens GN=HIST1H2BC PE=1 SV=4	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BD	P58876	Histone H2B type 1-D OS=Homo sapiens GN=HIST1H2BD PE=1 SV=2	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BJ	P06899	Histone H2B type 1-J OS=Homo sapiens GN=HIST1H2BJ PE=1 SV=3	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BK	O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BL	Q99880	Histone H2B type 1-L OS=Homo sapiens GN=HIST1H2BL PE=1 SV=3	1.54	2.40	chromatin organization, nucleosome assembly	
HIST1H2BM	Q99879	Histone H2B type 1-M OS=Homo sapiens GN=HIST1H2BM PE=1 SV=3	1.54	2.40	chromatin organization, nucleosome assembly	
HRG	P04196	Histidine-rich glycoprotein OS=Homo sapiens GN=HRG PE=1 SV=1	0.81	0.72	angiogenesis, blood coagulation, chemotaxis, defense response to fungus	
HSP90AA1	P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5	0.75	0.85	Fc-gamma receptor signaling pathway involved in phagocytosis, G2/M transition of mitotic cell cycle, cellular response to heat, chaperone mediated autophagy, chaperone mediated protein complex assembly, innate immune response	Yes
ICOSLG	O75144	ICOS ligand OS=Homo sapiens GN=ICOSLG PE=1 SV=2	0.75	0.82	B cell activation, T cell activation, adaptive immune response	Yes
IL1RAP	C9J9W1	Interleukin 1 receptor accessory protein OS=Homo sapiens GN=IL1RAP PE=4 SV=1	0.74	1.10	cytokine-mediated signaling pathway, immune response	
ING2	Q9H160	Inhibitor of growth protein 2 OS=Homo sapiens GN=ING2 PE=1 SV=1	0.85	0.34	chromatin modification, cell proliferation,	
ITGB1	P05556-5	Isoform Beta-1D of Integrin beta-1 OS=Homo sapiens GN=ITGB1	0.67	0.72	B cell differentiation, blood coagulation, cell migration, cell-matrix adhesion	
KRT3	P12035	Keratin, type II cytoskeletal 3 OS=Homo sapiens GN=KRT3 PE=1 SV=1	1.77	1.11	epithelial cell differentiation, intermediate filament cytoskeleton organization	
KRT15	P19012	Keratin, type I cytoskeletal 15 OS=Homo sapiens GN=KRT15 PE=1 SV=1	1.83	0.96	epidermis development, mitophagy in response to mitochondria depolarization	
KRT24	Q2M215	Keratin, type I cytoskeletal 24 OS=Homo sapiens GN=KRT24 PE=1 SV=1	1.87	1.04	epithelial cell differentiation	
KIT	P10721	Mast/stem cell growth factor receptor Kit OS=Homo sapiens GN=KIT PE=1 SV=1	0.79	0.58	Kit signaling pathway, T cell differentiation, MAPK cascade, actin cytoskeleton	Yes
LAMP1	P11279	Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3	0.71	0.76	Golgi to lysosome transport, autophagy, granzyme-mediated apoptotic signaling pathway, natural killer cell degranulation	Yes
LGALS1	P09382	Galectin-1 OS=Homo sapiens GN=LGALS1 PE=1 SV=2	0.97	0.65	T cell costimulation, apoptosis	
LUM	P51884	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	0.74	0.63	cartilage development, collagen fibril organization, extracellular matrix organization	
MCAM/CD146	P43121	Cell surface glycoprotein MUC18 OS=Homo sapiens GN=MCAM PE=1 SV=2	0.67	0.77	angiogenesis, cell adhesion, endothelial cell migration, vascular wound healing	Yes
MMP3	P08254	Stromelysin-1 OS=Homo sapiens GN=MMP3 PE=1 SV=2	1.48	1.48	extracellular matrix organization and disassembly, proteolysis, cell migration	Yes
MMRN2	Q9H8L6	Multimerin-2 OS=Homo sapiens GN=MMRN2 PE=1 SV=2	0.71	0.91	angiogenesis, cell migration, regulation of vascular endothelial growth factor receptor signaling pathway	
MRC1	P22897	Macrophage mannose receptor 1 OS=Homo sapiens GN=MRC1 PE=1 SV=1	1.23	1.63	cellular response to interferon-gamma, cellular response to lipopolysaccharide, receptor mediated endocytosis	
NCAM1	P13591-6	Isoform 6 of Neural cell adhesion molecule 1 OS=Homo sapiens GN=NCAM1	0.76	0.75	innate immune response, interferon-gamma-mediated signaling pathway, MAPK cascade	Yes
NEO1	Q92859	Neogenin OS=Homo sapiens GN=NEO1 PE=1 SV=2	0.81	0.75	cell adhesion, myoblast fusion	
PCOLCE	A4D2D2	Procollagen C-endopeptidase enhancer OS=Homo sapiens GN=PCOLCE PE=4 SV=1	0.74	0.75	peptidase activity, proteolysis	
PGK1	P00558	Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=1	1.32	1.33	Canonical glycolysis, epithelial cell	
PI16	Q6UXB8	Peptidase inhibitor 16 OS=Homo sapiens GN=PI16 PE=1 SV=1	0.69	0.54	peptidase activity, cell growth	
PI3	P19957	Elafin OS=Homo sapiens GN=PI3 PE=1 SV=3	1.05	1.83	endopeptidase activity	Already performed in CXCL9 paper
PLG	P00747	Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2	1.00	0.60	extracellular matrix organization and disassembly, blood coagulation, fibrinolysis	
PODXL	O00592	Podocalyxin OS=Homo sapiens GN=PODXL PE=1 SV=2	0.84	0.69	cell adhesion, leukocyte migration	
POSTN	B1ALD8	Periostin, osteoblast specific factor OS=Homo sapiens GN=POSTN PE=4 SV=1	0.72	0.69	extracellular matrix organization, regulation of Notch signaling pathway	Yes

PPIAL4A	Q9Y536	Peptidylprolyl cis-trans isomerase A-like 4A/B/C OS=Homo sapiens GN=PPIAL4A PE=1 SV=1	1.36	1.29	protein folding, protein peptidyl-prolyl isomerization	
PRDX2	P32119	Peroxisredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5	1.51	2.42	T cell proliferation, activation of MAPK activity, cellular response to oxidative stress	
PRG2	P13727	Bone marrow proteoglycan OS=Homo sapiens GN=PRG2 PE=1	1.35	1.25	innate immune response	
PSMA1	P25786-2	Isoform Long of Proteasome subunit alpha type-1 OS=Homo sapiens GN=PSMA1	1.24	1.32	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest, antigen processing and presentation of exogenous peptide antigen via MHC class I	
PTPRK	E9PGC5	Protein tyrosine phosphatase, receptor type, K OS=Homo sapiens GN=PTPRK PE=4 SV=1	0.96	0.70	Cell adhesion, cell migration	
PTPRS	Q13332-5	Isoform PTPS-F4-7 of Receptor-type tyrosine-protein phosphatase S OS=Homo sapiens GN=PTPRS	0.74	0.80	establishment of endothelial intestinal barrier	
PTX3	P26022	Pentraxin-related protein PTX3 OS=Homo sapiens GN=PTX3 PE=1 SV=3	1.04	1.64	inflammatory response, innate immune response, negative regulation of exo-alpha-sialidase activity, opsonization	
RRAGA	Q7L523	Ras-related GTP-binding protein A OS=Homo sapiens GN=RRAGA PE=1 SV=1	0.68	0.72	apoptosis, cell cycle arrest, cellular response to starvation, macroautophagy	
RRAGB	Q5VZM2	Ras-related GTP-binding protein B OS=Homo sapiens GN=RRAGB PE=1 SV=1	0.68	0.72	apoptosis, cell cycle arrest, cellular response to starvation, macroautophagy	
S100A9	P06702	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1	0.73	2.20	autophagy, cytokine production, chemokine production, innate immune response, neutrophils chemotaxis	
SAA1	E9PQD6	Serum amyloid A protein OS=Homo sapiens GN=SAA1 PE=3 SV=1	0.74	0.80	acute phase response, innate immune response, lymphocyte chemotaxis	
SAA2	G3V1D9	Serum amyloid A protein OS=Homo sapiens GN=SAA2 PE=3 SV=1	0.74	0.80	acute phase response, innate immune response, lymphocyte chemotaxis	
SDPR	O95810	Serum deprivation-response protein OS=Homo sapiens GN=SDPR PE=1 SV=3	1.52	1.28	Phospholipid binding, protein kinase C binding	
SELENBP1	Q13228	Selenium-binding protein 1 OS=Homo sapiens GN=SELENBP1 PE=1 SV=2	1.09	1.62	Protein transport, selenium binding	
SELL/CD62L	P14151	L-selectin OS=Homo sapiens GN=SELL PE=1 SV=2	0.75	0.75	blood coagulation, cell adhesion, leukocyte migration, inflammatory response	Yes
SELP/CD62P	Q5R345	Selectin P (Granule membrane protein 140kDa, antigen CD62) OS=Homo sapiens GN=SELP PE=2 SV=1	1.25	1.25	blood coagulation, cell adhesion, leukocyte migration, inflammatory response	Yes
SERPINA1	P01009	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	0.86	0.33	Acute phase response, COPII vesicle coating, ER to golgi vesicle-mediated transport, blood coagulation	
SERPINA2	P20848	Putative alpha-1-antitrypsin-related protein OS=Homo sapiens GN=SERPINA2 PE=5 SV=1	1.21	0.46	endopeptidase activity	
SERPINA5	G3V264	Serpin peptidase inhibitor, clade A (alpha-1 antitrypsin), member 5 OS=Homo sapiens GN=SERPINA5 PE=4	0.73	1.10	blood coagulation, lipid transport, endopeptidase activity	
SERPINA6	P08185	Corticosteroid-binding globulin OS=Homo sapiens GN=SERPINA6 PE=1 SV=1	0.73	0.70	glucocorticoid metabolic process, endopeptidase activity	
SERPINA7	P05543	Thyroxine-binding globulin OS=Homo sapiens GN=SERPINA7 PE=1 SV=2	0.78	0.71	aging, endopeptidase activity, response to corticosterone	
SERPINF2	B4E1B7	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2 OS=Homo sapiens GN=SERPINF2 PE=2 SV=1	0.72	0.87	Acute phase response, blood coagulation, blood vessel morphogenesis, collagen fibril organization, fibrinolysis	
SLIT2	O94813	Slit homolog 2 protein OS=Homo sapiens GN=SLIT2 PE=1 SV=1	0.64	0.83	Roundabout signaling pathway, apoptosis, cell migration involved in sprouting angiogenesis, chemotaxis	
SOD3	P08294	Extracellular superoxide dismutase [Cu-Zn] OS=Homo sapiens GN=SOD3 PE=1 SV=2	0.75	0.95	oxidation-reduction process, response to hypoxia	
SPP1/OPN	P10451-3	Isoform C of Osteopontin OS=Homo sapiens GN=SPP1	0.87	0.75	biomineral tissue development, cell adhesion, cellular response to fluid shear stress, extracellular matrix organization and disassembly, inflammatory response, neutrophils chemotaxis, osteoblast	Yes
SPP2	Q13103	Secreted phosphoprotein 24 OS=Homo sapiens GN=SPP2 PE=1 SV=1	0.84	0.68	Bone remodeling	
SYNE1	E9PEL9	Spectrin repeat containing, nuclear envelope 1 OS=Homo sapiens GN=SYNE1 PE=4 SV=1	0.79	0.68	Golgi organization, cytoskeletal anchoring at nuclear membrane	
TACC2	O95359	Transforming acidic coiled-coil-containing protein 2 OS=Homo sapiens GN=TACC2 PE=1 SV=3	0.71	0.77	Astral microtubule organization, cell proliferation	
TGFBI	Q15582	Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFBI PE=1 SV=1	1.25	1.25	angiogenesis, cell adhesion, cell proliferation, chondrocyte differentiation, extracellular matrix organization	Yes
TMSB10	P63313	Thymosin beta-10 OS=Homo sapiens GN=TMSB10 PE=1 SV=2	1.54	1.25	actin filament organization	
TMSB4X	P62328	Thymosin beta-4 OS=Homo sapiens GN=TMSB4X PE=1 SV=2	1.56	1.27	actin filament organization, blood coagulation, platelet degranulation	
TMSL3	A8MW06	Thymosin beta-4-like protein 3 OS=Homo sapiens GN=TMSL3 PE=2 SV=1	1.56	1.27	actin filament organization	
TMSL4	Q5T4B6	HCG1780554 OS=Homo sapiens GN=TMSL4 PE=4 SV=1	1.56	1.27	actin filament organization	

TNFRSF10C/T RAILR3	F5H3Y5	TNFRSF10Cprotein OS=Homo sapiens GN=TNFRSF10C PE=4 SV=1	1.28	1.25	extrinsic apoptotic signaling pathway via death domain receptors, immune response, tumor necrosis factor-mediated signaling	Yes
TNXB VCAN	E7EPO1 P13611	Tenascin XB OS=Homo sapiens GN=TNXB PE=4 SV=1 Versican core protein OS=Homo sapiens GN=VCAN PE=1 SV=3	0.75 1.04	0.82 2.60	cell-matrix adhesion carbohydrate metabolic process, cell recognition, chondroitin sulfate catabolic process, extracellular matrix organization, actin filament capping, actin filament depolymerization, cellular response to epidermal growth factor stimulus	
VIL1	P09327	Villin-1 OS=Homo sapiens GN=VIL1 PE=1 SV=4	0.62	0.67		
VNN1	O95497	Pantetheinase OS=Homo sapiens GN=VNN1 PE=1 SV=2	1.14	0.70	acute inflammatory response, chronic inflammatory response, pantothenate metabolic process, positive regulation of T cell differentiation in thymus. response to complement activation, alternative pathway,	
VSIG4	O9Y279	V-set and immunoglobulin domain-containing protein 4 OS=Homo sapiens GN=VSIG4 PE=1 SV=1	1.15	1.77	negative regulation of T cell proliferation, negative regulation of interleukin-2 production	

Table S2: Twenty-two proteins selected from the proteomics discovery

Protein	Description	Commercial ELISA provider	Plasma dilution	LLOD	ULOD
ACAN	aggrecan	R&D DuoSet	1/3	125 pg/mL	8000 pg/mL
CD44	CD44 molecule	eBioscience	1/60	125 pg/mL	4000 pg/mL
CDH5/VE cadherin	cadherin 5, type 2 (vascular endothelium)	R&D Quantikine	1/100	1.56 ng/mL	100 ng/mL
COMP	cartilage oligomeric matrix protein	R&D Quantikine	1/100	0.16 ng/mL	10 ng/mL
ECM1	extracellular matrix protein 1	SinoBiological	1/2000	0.023 ng/mL	1.5 ng/mL
ENG/CD105	endoglin	R&D DuoSet	1/50	125 pg/mL	8000 pg/mL
FAP	fibroblast activation protein, alpha	R&D DuoSet	1/100	62.5 pg/mL	4000 pg/mL
FCN3	ficolin (collagen/fibrinogen domain containing) 3	Hycult	1/150	7.8 ng/mL	500 ng/mL
FN1	fibronectin 1	eBioscience	1/20000	0.31 ng/mL	20 ng/mL
HSP90a	Heat shock protein 90 alpha	Enzo Life Science	1/25	0.063 ng/mL	4 ng/mL
ICOSLG	inducible T-cell co-stimulator ligand	SinoBiological	1/30	0.063 ng/mL	4 ng/mL
C-KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Cell Sciences	1/15	0.31 ng/mL	10 ng/mL
LAMP1	lysosomal-associated membrane protein 1	SinoBiological	1/100	15.6 pg/mL	1000 pg/mL
MCAM/CD146	melanoma cell adhesion molecule	SinoBiological	1/250	0.078 ng/mL	5 ng/mL
MMP3	matrix metalloproteinase 3 (stromelysin 1)	R&D DuoSet	1/25	31.2 pg/mL	2000 pg/mL
NCAM1/CD56	neural cell adhesion molecule 1	R&D DuoSet	1/200	78.1 pg/mL	5000 pg/mL
POSTN	periostin, osteoblast-specific factor	R&D DuoSet	1/25	0.36 ng/mL	24 ng/mL
SELL/CD62L	selectin L	R&D DuoSet	1/200	78.1 pg/mL	5000 pg/mL
SELP	selectin P	R&D DuoSet	1/50	125 pg/mL	8000 pg/mL
SPP1/OPN	osteopontin/secreted phosphoprotein 1	R&D DuoSet	1/25	62.5 pg/mL	4000 pg/mL
TGFBI	transforming growth factor, beta-induced, 68 kDa	R&D DuoSet	1/1000	62.5 pg/mL	4000 pg/mL
TNFRSF10C/TRAILR3	tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	R&D DuoSet	1/25	62.5 pg/mL	4000 pg/mL

LLOD, lower limit of detection; ULOD, upper limit of detection.

Table S3. Biomarker panel according to cGVHD status and particular organ involvement in verification cohort 1 and 2.

	Organ	N			Median (range)			P-value			
		Control (No cGVHD)	cGVHD +		Control (No cGVHD)	cGVHD+		Kruskal- Wallis	cGVHD+		
			Organ -	Organ +		Organ -	Organ +		Organ + vs. Organ -	Organ – vs. Control	Organ + vs. Control
Verification cohort 1	Skin	33	65	113	10.0 (7.5-13.7)	12.7 (7.8-19.1)	12.8 (8.8-17.3)	<0.0001	0.58	<0.0001	<0.0001
	GI	33	121	57	10.0 (7.5-13.7)	12.6 (8.8-19.1)	13.3 (7.8-17.3)	<0.0001	0.07	<0.0001	<0.0001
	Mouth	33	67	111	10.0 (7.5-13.7)	12.6 (9.0-19.1)	12.8 (7.8-17.3)	<0.0001	0.99	<0.0001	<0.0001
	Eye	33	97	81	10.0 (7.5-13.7)	12.9 (8.8-19.1)	12.6 (7.8-17.3)	<0.0001	0.37	<0.0001	<0.0001
	Lung	33	61	116	10.0 (7.5-13.7)	12.8 (7.8-17.3)	12.7 (8.8-19.1)	<0.0001	0.46	<0.0001	<0.0001
	Liver	33	97	80	10.0 (7.5-13.7)	12.5 (7.8-16.6)	13.1 (9.1-19.1)	<0.0001	0.003	<0.0001	<0.0001
Verification cohort 2	Skin	93	39	44	2.04 (0.32-3.97)	2.85 (0.19-4.69)	3.08 (-0.34-4.84)	<0.0001	0.83	<0.0001	<0.0001
	GI	93	62	22	2.04 (0.32-3.97)	2.92 (-0.34-4.84)	3.34 (0.19-4.92)	<0.0001	0.14	<0.0001	0.0003
	Mouth	93	37	48	2.04 (0.32-3.97)	2.95 (1.11-4.84)	2.97 (-0.34-4.92)	<0.0001	0.46	<0.0001	<0.0001
	Eye	93	51	33	2.04 (0.32-3.97)	3.00 (-0.34-4.69)	2.95 (1.85-4.92)	<0.0001	0.19	<0.0001	<0.0001
	Lung	93	42	40	2.04 (0.32-3.97)	3.01 (1.30-4.92)	2.89 (-0.34-4.56)	<0.0001	0.51	<0.0001	<0.0001
	Liver	93	41	41	2.04 (0.32-3.97)	2.82 (-0.34-4.92)	3.02 (1.30-4.56)	<0.0001	0.35	0.0001	<0.0001