SUPPLEMENTARY INFORMATION

A genomic and clinical prognostic index for hepatitis C-related early-stage cirrhosis that predicts clinical deterioration

Lindsay Y. King[1], Claudia Canasto-Chibuque[2], Kara Johnson[1], Shun Yip[2], Xintong Chen[2], Kensuke Kojima[2], Manjeet Deshmukh[2], Anu Venkatesh[2], Poh Seng Tan[2,3], Xiaochen Sun[2], Augusto Villanueva[4], Angelo Sangiovanni[5], Venugopalan Nair[6], Milind Mahajan[7], Masahiro Kobayashi[8], Hiromitsu Kumada[8], Massimo Iavarone[5], Massimo Colombo[5], Maria Isabel Fiel[9], Scott L. Friedman[2], Josep M. Llovet[2,10,11], Raymond T. Chung[1], Yujin Hoshida[2]

[1]Liver Center and Gastrointestinal Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, U.S.
[2]Liver Cancer Program, Tisch Cancer Institute, Division of Liver Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, U.S.
[3]Division of Gastroenterology and Hepatology, University Medicine Cluster, National University Health System, Singapore
[4]Institute of Liver Sciences, King's College London, U.K.
[5]M. & A. Migliavacca Center for Liver Disease and 1st Division of Gastroenterology, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Italy
[6]Department of Neurology, Icahn School of Medicine at Mount Sinai, U.S.
[7]Institute of Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, U.S.
[8]Department of Hepatology, Toranomon Hospital, Japan
[9]Department of Pathology, Icahn School of Medicine at Mount Sinai, U.S.
[10]HCC Translational Research Laboratory, Barcelona Clinic Liver Cancer Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer Centro de Investigaciones en Red de Enfermedades Hepáticas y Digestivas, Hosptial Clínic Barcelona, Catalonia, Spain
[11]Institució Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Catalonia, Spain

Signature gene reduction

The genes in the 186-gene signature were selected to be correlated with clinical outcome based on Cox score: a larger positive Cox score indicates stronger association with poor outcome, and a smaller negative Cox score indicates stronger association with good outcome.[1] The signature genes were reduced based on the Cox score in the training cohort, for which both genome-wide microarray and nCounter datasets were generated, and consistency of the prediction between reduced and full signatures was evaluated. First, subsets of the signature genes with larger absolute Cox scores calculated in the original genome-wide microarray dataset were selected based on the following cut-offs: 2.3, 2.5, and 3.0 (the smallest absolute Cox score was 2.13), which yielded 169, 116, and 32, signature genes, respectively. The best prediction consistency was observed at the Cox score cut-off of 3.0 (32-gene signature) (**Supplementary figure 3A**, **Supplementary table 4**). Alternatively, subsets of the signature genes were selected based on absolute Cox scores calculated in the nCounter dataset. At Cox score cut-offs of 1.0, 1.5, 2.0, 2.5, and 3.0, 97, 52, 32, 11, and 5 signature genes were selected, respectively. The best prediction consistency was observed at the cut-off of 2.5 (11-gene signature) (**Supplementary figure 3B**, **Supplementary table 5**). Association of the reduced gene signatures and overall death was evaluated by log-rank test (**Supplementary figure 4**). The prognostic index was calculated for each of the reduced gene signatures, and association with the clinical outcomes was verified using Cox regression modeling (**Supplementary table 6** and **7**).

REFERENCES

1. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 2008;359:1995-2004.

Supplementary table 1

Gene-signature-based prognostic prediction in paired serial biopsies and multiple anatomical locations in explanted liver.

Case	Clinical diagnosis	Type of sample	Necroinflammatory grade	Fibrosis stage	Interval between paired biopsies	186-gene signature prediction
#1	Chronic hepatitis	Core needle biopsy #1	5	2	7.5 years	Good
		Core needle biopsy #2	5	2		Good
#2	Chronic hepatitis	Core needle biopsy #1	5	0	4 months	Intermediate
		Core needle biopsy #1*	5	0		Intermediate
		Core needle biopsy #2	5	0		Good
#3	Cirrhosis	Core needle biopsy #1	6	5	1 month	Poor
		Core needle biopsy #2	6	5		Intermediate
#4	Cirrhosis**	Explanted liver, right. lobe	2	0	n.a.	Poor
		Explanted liver, left lobe	2	0		Poor

*Technical replicate of RNA sample.

**Specimens were obtained from allograft with rejection.

Necroinflammatory grade (max possible score: 18) and fibrosis stage (max possible score: 6) are based on Ishak, et al. J Hepatol 22;696-699,1995.

Supplementary table 2
Prognostic association of the prognostic index in the training cohort (Cox regression).

Variable	Hazard ratio (95% confidence interval)	p-value
Hepatic decompensation (n=71, 34%)		
Intermediate risk group	1.67 (0.85-3.27)	0.14
High risk group	2.71 (1.42-5.18)	0.003
Overall death (n=66, 31%)		
Intermediate risk group	1.72 (0.78-3.81)	0.18
High risk group	6.00 (2.85-12.64)	<0.001
Hepatocellular carcinoma (n=65, 30%)		
Intermediate risk group	2.06 (1.03-4.11)	0.04
High risk group	3.31 (1.62-6.77)	0.001
Progression of Child-Pugh class (n=66, 31%)		
Intermediate risk group	2.09 (0.90-4.81)	0.08
High risk group	6.70 (3.04-14.75)	<0.001

Hazard ratios were computed by comparing to low risk group. Hepatic decompensation is the composite of variceal bleeding, ascites, and hepatic encephalopathy.

Supplementary table 3

Comparison of clinical demographics with previously reported prognostic studies for hepatitis C-related Child-Pugh class A/compensated cirrhosis

		Individual studies describing hepatitis C-related Child-Pugh A/compensated cirrhosis								*Reviews including other etiologies*			
	Our validation cohort	Our training cohort *Gastroenterology 2013;144:1024-30*	Degos, et al. *Gut 2000;47:131-6*	Fattovich, et al. *Gastroenterology 1997;112:463-72*	Fattovich, et al. *Am J Gastroenterol 2002;97:2886-95*	Bruno, et al. *Am J Gastroenterol 2009;104:1147-58*	Ikeda, et al. *Hepatology 1993;18:47-53*	Hu, et al. *Hepatology 1999;29:1311-6*	Serfaty, et al. *Hepatology 1998;27:1435-40*	D'Amico, et al. *J Hepatol 2006;44:217-31*	Fattovich, et al. *Gastroenterology 2004;127:S35-50*		de Franchis, et al. *J Hepatol 2005;43:167-76*
No. of patients	145	216	416	384	136	352	349	112	103	20-114 studies	1284 (13 studies)	626 (7 studies)	1649 (2 studies)
Age (yr) - median (range)	49 (45-55)	59 (34-75)	57 (IQR:46-64)	mean 54 (19-78)	58 (22-79)	59 (21-70)	55 (25-84)	51 (30-78)	55	-	56	59	-
Sex - male (%)	107 (74%)	116 (54%)	240 (58%)	223 (58%)	81 (61%)	180 (51%)	234 (67%)	56 (50%)	72 (70%)	-	58%	58%	-
Ethnicity	Caucasian (85%), Black (6%), Hispanic (7%)	Caucasian	-	Caucasian (96%), Asian (3%), African (1%)	Caucasian	na	Asian	Caucasian (58%), Hispanic (22%), Asian (16%), African (4%)	-	-	-	Asian	-
Geographic site	United States	Europe	Europe	Europe	Europe	Europe	Japan	United States	Europe	-	Europe/United States	Japan	-
Severity of cirrhosis - Child-Pugh A (%)	83%, Compensated (100%)	100%	100%	Compensated (100%)	100%	100%	Compensated (94%)	Compensated (100%)	91%	100%	Compensated	Compensated	-
Etiology - hepatitis C (%)	100%	100%	100%	100%	100%	100%	100%	100%	100%	median 35 % (range: 0-100%)	100%	100%	-
Hepatitis C virus genotype 1b (%)	93 (72%), genotype 1	122 (58%)	-	-	-	221 (63%)	-	59 (53%)	49 (48%)	-	-	-	-
History of interferon treatment	-	101 (47%)	223 (54%)	205 (53%)	0%	194 (55%)	-	49 (44%)	59 (57%)	-	-	-	-
Gastric/esophageal varices	-	52 (25%)	175 (42%)	79 (41%)	44 (52%)	51 (14%)	-	-	-	-	-	-	-
Incidence of death - no. (%)	50 (34%)	66 (31%)	83 (20%)	51 (13%)	52 (38%)	158 (45%)	126 (36%)	15 (13%)	16 (16%)	median 36% (range: 11-100%)	-	-	-
- Annual rate	1.7%*	2.1%*	3.3%*	1.9%	3.5%*	3.0%	3.5%* (with other etiologies)	3.4%	3.5%*	-	-	-	-
- Annual rate in Baveno IV stage 1**	-	1.8%*	-	-	-	-	-	-	-	-	-	-	1.0%
- Annual rate in Baveno IV stage 2**	-	3.5%*	-	-	-	-	-	-	-	-	-	-	3.4%
- 1yr survival rate	100%	99%	-	-	-	-	98% (with other etiologies)	-	-	94% (range: 75-100%)	-	-	-
- 2yr survival rate	98%	99%	-	-	-	-	96% (with other etiologies)	-	96%	90% (range: 70-100%)	-	-	-
- Liver-related death (%)	64%	72%	77%	70%	67%	70%	83% (with other etiologies)	95%	94%	-	-	-	-
Incidence of hepatic decompensation - no. (%)	45 (31%)	71 (34%)	-	65 (18%)	49 (36%)	131 (37%)	-	24 (21%)	45 (44%)	-	-	-	-
- Annual rate	3.9%*	3.3%*			5.3%	3.5%	-	4.4%		-	-	-	-
- Ascites - no.	34	62	-	31	-	66	-	10	-	-	-	-	-
- Bleeding - no.	17	22	-	14	-	22	-	5	-	-	-	-	-
- Encephalopathy - no.	27	10	-	5	-	21	-	4	-	-	-	-	-
Incidence of hepatocellular carcinoma - no. (%)	21 (14%)	65 (30%)	60 (14%)	29 (8%)	23 (17%)	109 (31%)	154 (44%)	9 (8%)	11 (11%)	-	-	-	-
- Annual rate	1.3%*	2.9%	2.9%*	1.4%	2.5%	2.9%	4.8%*	2.0%	3.3%	-	3.7%	7.1%	-
Follow-up time (yr) - median (range)	8.0 (1.2-22.9)	9.8 (0.5-22.6)	5.7 (0.3-16.6)	5.1 (0.5-12.8)	6.8 (0.5-15.9)	14.4 (0.9-19.5)	5.8 (2.0-17.0)	mean 4.5 (2.0-7.7)	3.3 (0.5-6.0)	median 2.6 (range: 0.5-14)	-	-	-

*Annual rate was calculated using Declining Exponential Approximation of Life Expectancy (DEALE) based on cumulative 5-year incidence [Beck, et al. Am J Med 73;889-97,1982].

**de Franhis, J Hepatol 2005;43(1):167-76

Supplementary table 4
Reduced 186-gene signature (32-gene signature) based on Cox score calculated in genome-wide microarray dataset.

Entrez gene ID	Gene symbol	Gene name	Correlated with	Cox score
2488	FSHB	follicle stimulating hormone, beta polypeptide	poor outcome	4.80
6456	SH3GL2	SH3-domain GRB2-like 2	poor outcome	4.21
23029	RBM34	RNA binding motif protein 34	poor outcome	4.19
23397	NCAPH	non-SMC condensin I complex, subunit H	poor outcome	4.02
1950	EGF	epidermal growth factor (beta-urogastrone)	poor outcome	3.97
7204	TRIO	triple functional domain (PTPRF interacting)	poor outcome	3.90
1293	COL6A3	collagen, type VI, alpha 3	poor outcome	3.87
3983	ABLIM1	actin binding LIM protein 1	poor outcome	3.86
3680	ITGA9	integrin, alpha 9	poor outcome	3.81
4922	NTS	neurotensin	poor outcome	3.78
5055	SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	poor outcome	3.69
4316	MMP7	matrix metallopeptidase 7 (matrilysin, uterine)	poor outcome	3.59
5593	PRKG2	protein kinase, cGMP-dependent, type II	poor outcome	3.44
9170	EDG4	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 4	poor outcome	3.40
4843	NOS2A	nitric oxide synthase 2A (inducible, hepatocytes)	poor outcome	3.33
2043	EPHA4	EPH receptor A4	poor outcome	3.25
6672	SP100	SP100 nuclear antigen	poor outcome	3.19
2326	FMO1	flavin containing monooxygenase 1	poor outcome	3.04
2877	GPX2	glutathione peroxidase 2 (gastrointestinal)	poor outcome	3.02
9252	RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	good outcome	-3.00
5313	PKLR	pyruvate kinase, liver and RBC	good outcome	-3.01
27346	TMEM97	transmembrane protein 97	good outcome	-3.06
5502	PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	good outcome	-3.07
5691	PSMB3	proteasome (prosome, macropain) subunit, beta type, 3	good outcome	-3.09
5771	PTPN2	protein tyrosine phosphatase, non-receptor type 2	good outcome	-3.12
151	ADRA2B	adrenergic, alpha-2B-, receptor	good outcome	-3.19
6296	ACSM3	acyl-CoA synthetase medium-chain family member 3	good outcome	-3.21
3612	PFKFB1	inositol(myo)-1(or 4)-monophosphatase 1	good outcome	-3.22
5207	IMPA1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	good outcome	-3.22
6018	RLF	rearranged L-myc fusion	good outcome	-3.23
7276	TTR	transthyretin (prealbumin, amyloidosis type I)	good outcome	-3.27
223	ALDH9A1	aldehyde dehydrogenase 9 family, member A1	good outcome	-3.34

Supplementary table 5
Reduced 186-gene signature (11-gene signature) based on Cox score calculated in nCounter dataset.

Entrez gene ID	Gene symbol	Gene name	Correlated with	Cox score
1293	COL6A3	collagen, type VI, alpha 3	poor outcome	3.87
8870	IER3	immediate early response 3	poor outcome	2.98
165	AEBP1	AE binding protein 1	poor outcome	2.67
6363	CCL19	chemokine (C-C motif) ligand 19	poor outcome	2.23
10458	BAIAP2	BAI1-associated protein 2	good outcome	-2.31
1486	CTBS	chitobiase, di-N-acetyl-	good outcome	-2.54
157567	ANKRD46	ankyrin repeat domain 46	good outcome	-2.54
6718	AKR1D1	aldo-keto reductase family 1, member D1	good outcome	-2.76
3479	IGF1	insulin-like growth factor 1 (somatomedin C)	good outcome	-2.84
25828	TXN2	thioredoxin 2	good outcome	-2.90
27346	TMEM97	transmembrane protein 97	good outcome	-3.06

Supplementary table 6
Prognostic association of the prognostic index based on reduced 186-gene signature (32-gene signature). (Cox regression).

Variable	Hazard ratio (95% confidence interval)	p-value
Hepatic decompensation (n=45, 31%)		
Intermediate risk group	2.46 (1.14-5.30)	0.02
High risk group	5.85 (2.65-12.92)	<0.001
Overall death (n=50, 34%)		
Intermediate risk group	1.93 (0.97-3.85)	0.06
High risk group	3.42 (1.63-7.19)	0.001
Liver-related death (n=32, 22%)		
Intermediate risk group	1.88 (0.74-4.77)	0.18
High risk group	5.07 (2.03-12.65)	<0.001
All liver-related adverse events (n=59, 41%)		
Intermediate risk group	2.71 (1.42-5.21)	0.003
High risk group	4.18 (2.02-8.68)	<0.001

Hazard ratios were computed by comparing to low risk group. Hepatic decompensation is the composite of variceal bleeding, ascites, and hepatic encephalopathy. The composite outcome incorporates hepatic decompensation, HCC, and liver-related death.

Supplementary table 7
Prognostic association of the prognostic index based on reduced 186-gene signature (11-gene signature). (Cox regression).

Variable	Hazard ratio (95% confidence interval)	p-value
Hepatic decompensation (n=45, 31%)		
Intermediate risk group	3.02 (1.38-6.60)	0.006
High risk group	7.24 (3.20-16.37)	<0.001
Overall death (n=50, 34%)		
Intermediate risk group	1.84 (0.93-3.65)	0.08
High risk group	3.67 (1.79-7.53)	<0.001
Liver-related death (n=32, 22%)		
Intermediate risk group	2.01 (0.79-5.08)	0.14
High risk group	5.87 (2.357-14.62)	<0.001
All liver-related adverse events (n=59, 41%)		
Intermediate risk group	3.21 (1.66-6.20)	<0.001
High risk group	5.12 (2.43-10.76)	<0.001

Hazard ratios were computed by comparing to low risk group. Hepatic decompensation is the composite of variceal bleeding, ascites, and hepatic encephalopathy. The composite outcome incorporates hepatic decompensation, HCC, and liver-related death.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure 1

Probability of clinical outcomes analyzed in the validation cohort: (A) hepatic decompensation, (B) overall death, (C) liver-related death, and (D) all liver-related adverse events..

Supplementary figure 2

Association of the 186-gene signature-based prediction with overall death. P-value was calculated by log-rank test.

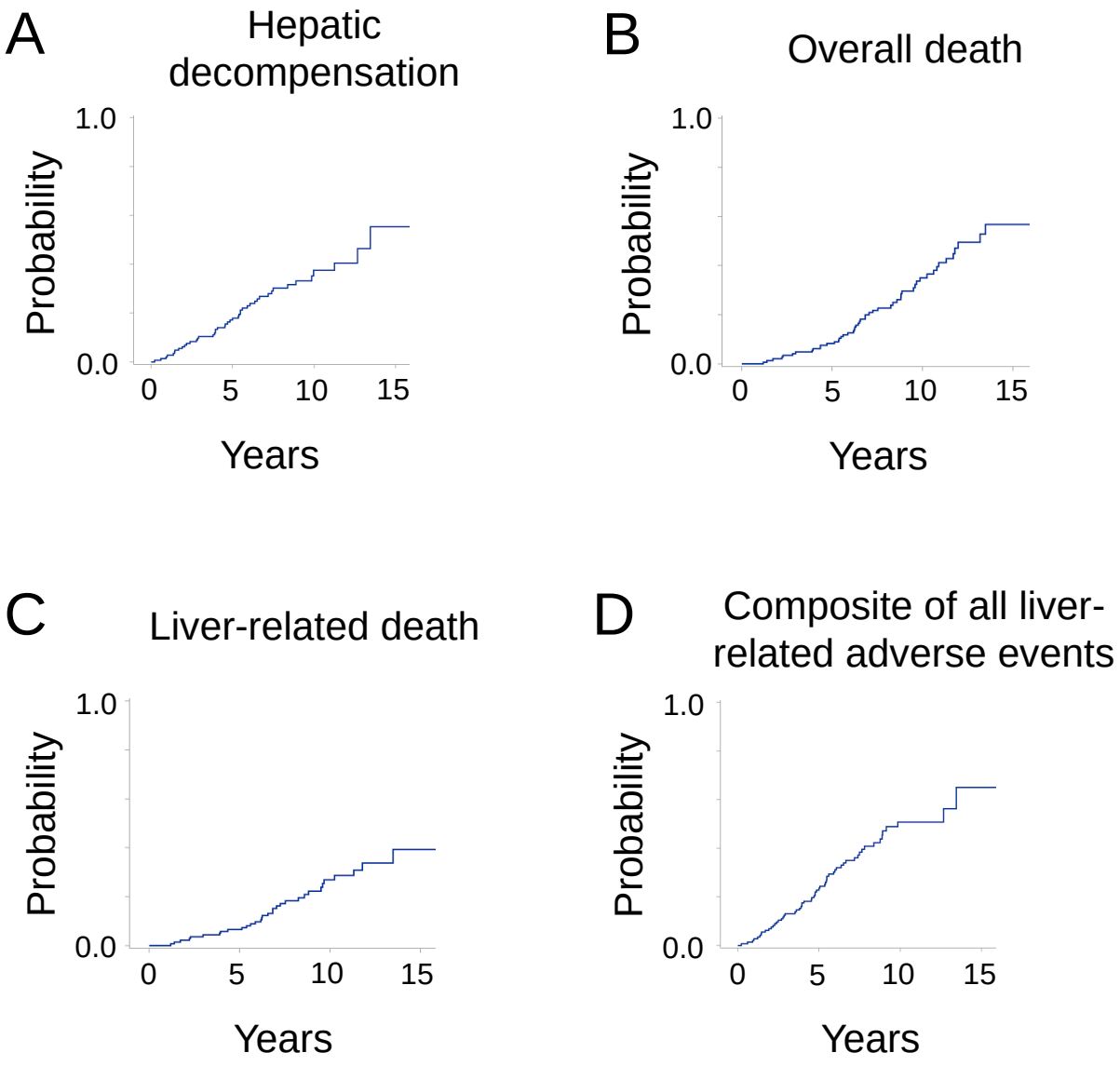
Supplementary figure 3

Consistency of prediction results between full and reduced gene signatures (training cohort). (A) Signature gene reduction based on Cox scores calculated in genome-wide microarray dataset. (B) Signature gene reduction based on Cox scores calculated in nCounter dataset.

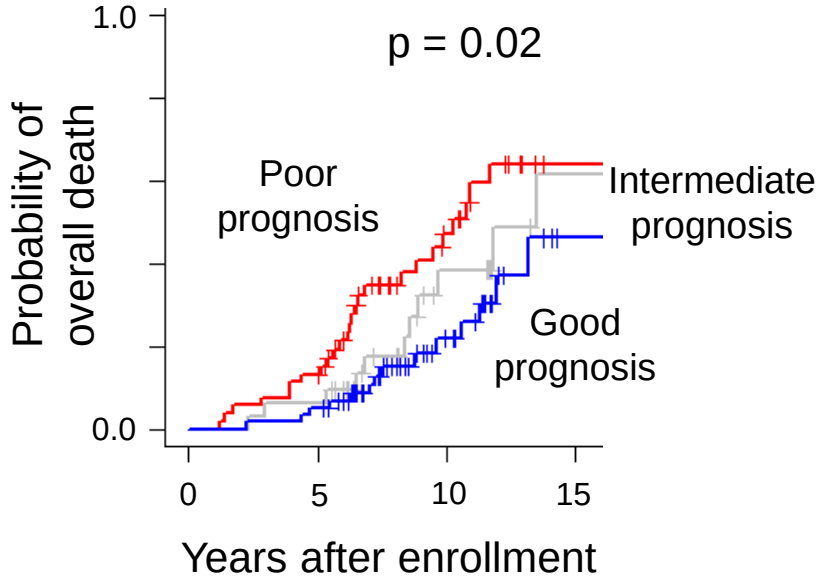
Supplementary figure 4

Association of reduced gene signature-based prediction and overall death (validation cohort). (A) Prediction based on the 32-gene signature. (B) Prediction based on the 11-gene signature. P-values were calculated by log-rank test.

Supplementary figure 1

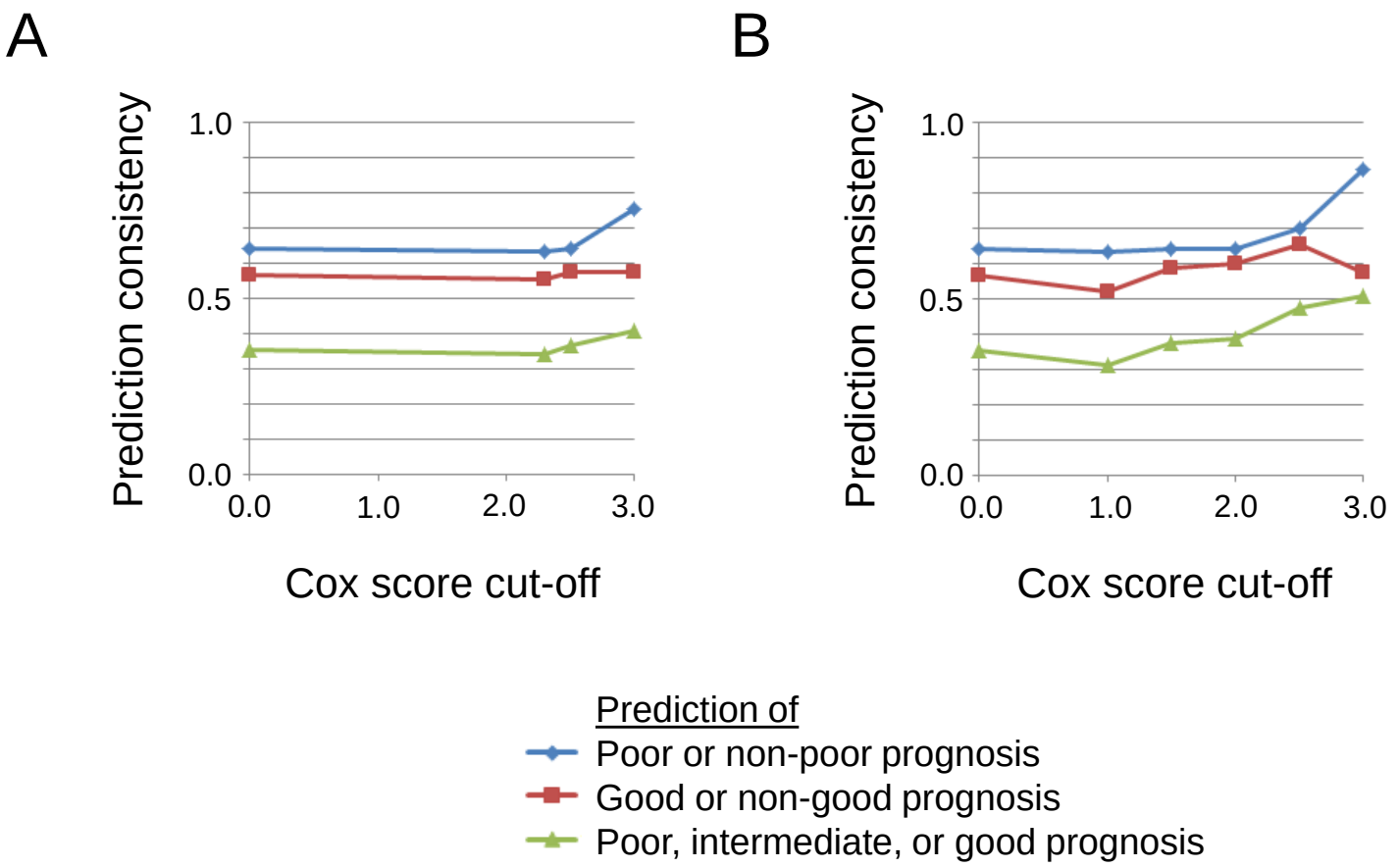


Supplementary figure 2



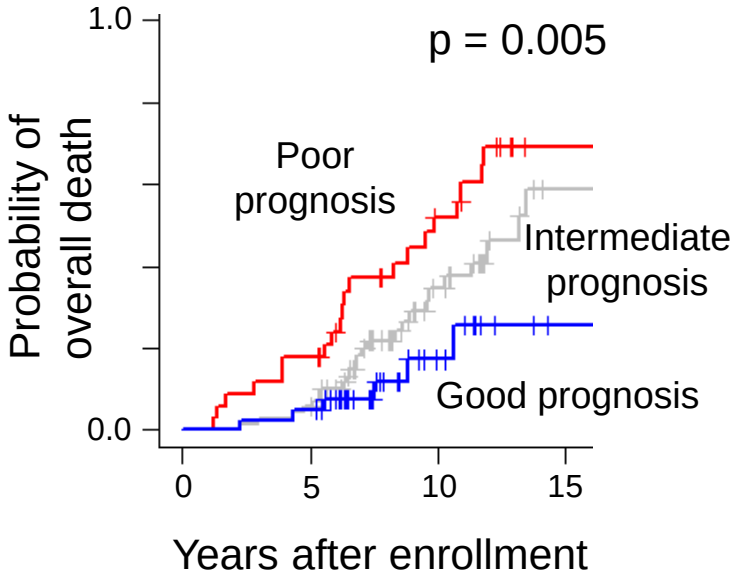
No. at risk				
Poor prognosis	53	46	15	1
Intermediate prognosis	32	30	10	3
Good prognosis	60	57	21	3

Supplementary figure 3



Supplementary figure 4

A



B