Supplementary Online Content

Cao Y, Liu H, Li H, et al. Association of O^6-methylguanine-DNA methyltransferase protein expression with postoperative prognosis and adjuvant chemotherapeutic benefits among patients with stage II or III gastric cancer. *JAMA Surg.* Published online September 13, 2017. doi:10.1001/jamasurg.2017.3120

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Construction of Tissue Microarray

The tissue microarrays (TMAs) are from one original patient cohort of 496 patients from Zhongshan Hospital, Fudan University. However, 468 of the 496 patients have integrated data. In this study, we used the TMAs manufactured by Shanghai Outdo Biotech Co., Itd. Tissue cylinders which were 2 mm in diameter were punched out from the targeted area of each tissue block and transferred into a recipient block with the use of a TMA instrument. Subsequently, 4-μm thick sections were consecutively sliced from each of the TMA blocks. As to each TMA block, one of the sections was stained with H&E for histological verification so as to ensure the arrayed tumor tissues were adequately established. Qualified samples were defined as those where the tumor tissue accounted for more than 10% of the core area. Sections were then placed on microscope slides for immunohistochemistry.

eMethods 2. Immunohistochemistry

The TMA slides were baked at 60℃ for 6 hours, deparaffinized in xylene (three times, 15 min each, room temperature) and rehydrated in graded alcohol. Endogenous peroxidase was blocked with 3% H_2O_2 in methanol at 37℃ for 30 minutes. Next, the slides were immersed in 0.01 M citrate buffer (pH 6.0), cooked for antigen retrieval and then incubated with 10% normal goat serum at 37℃ to reduce nonspecific reactions. Subsequently, mouse monoclonal antibody to human MGMT (1:300 dilution, ab39253, Abcam, Cambridge, UK) was applied to incubate the slides overnight at 4℃. After rinsing 3 times with 0.01 M phosphate buffer (pH = 8.0), the slides were incubated with secondary antibody for 20 minutes at 37℃ and stained with the use of diaminobenzidine (DAB)-H_2O_2. Ultimately, the TMA slides were counterstained with hematoxylin, dehydrated, and mounted with a coverslip and neutral resins. Fifteen dots of the 468 patients with integrated data were lost after immunohistochemistry.

eTable 1. Relationship Between MGMT Expression and Clinical Characteristics

Factors	Discovery Data Set				Validation Data Set		
	Negative	**Positive**	***P*-value**		**Negative**	**Positive**	***P*-value**
All patients	65	135			89	156	
Age(years)[a]			**0.045**				0.56
Median (IQR)	61 (57-68.5)	61 (54-70)			59 (52-67)	58 (50-68.75)	
Sex			0.85				0.33
Women	17	37			31	45	
Men	48	98			58	111	
Localization			0.18				0.55
Proximal	25	35			17	36	
Middle	7	21			11	24	
Distal	33	79			61	96	
Tumor size(cm)[a]			0.21				0.18
Median (IQR)	3.5 (2-4.5)	3.5 (2-4.5)			3.5 (2.75-5)	3.25 (2-5)	
Differentiation			0.34				0.54
Differentiated	16	42			26	40	
Undifferentiated	49	93			63	116	
Lauren classification			0.18				0.71
Intestinal type	39	94			58	98	
Diffuse type	26	41			31	58	
T classification			0.59				0.14
T1	13	33			12	26	
T2	5	17			14	27	
T3	13	23			24	23	
T4	34	62			39	80	
N classification			0.10				0.31
N0	18	62			28	66	
N1	9	12			13	16	
N2	17	29			17	22	
N3	21	32			31	52	
TNM stage			**0.01**				0.57
I	13	39			19	41	
II	10	39			19	36	
III	42	57			51	79	
Adjuvant chemotherapy[b]			0.90				0.81

No	30	61			34	62	
Yes	35	74			55	94	

Abbreviations: MGMT= O^6-methylguanine-DNA methyltransferase; TNM = Tumor-Node-Metastasis; *P*-value < 0.05 marked in bold font shows statistical significance; Negative and Positive refers to MGMT-negative and MGMT-positive, respectively.

[a]Modeled as a continuous variable.

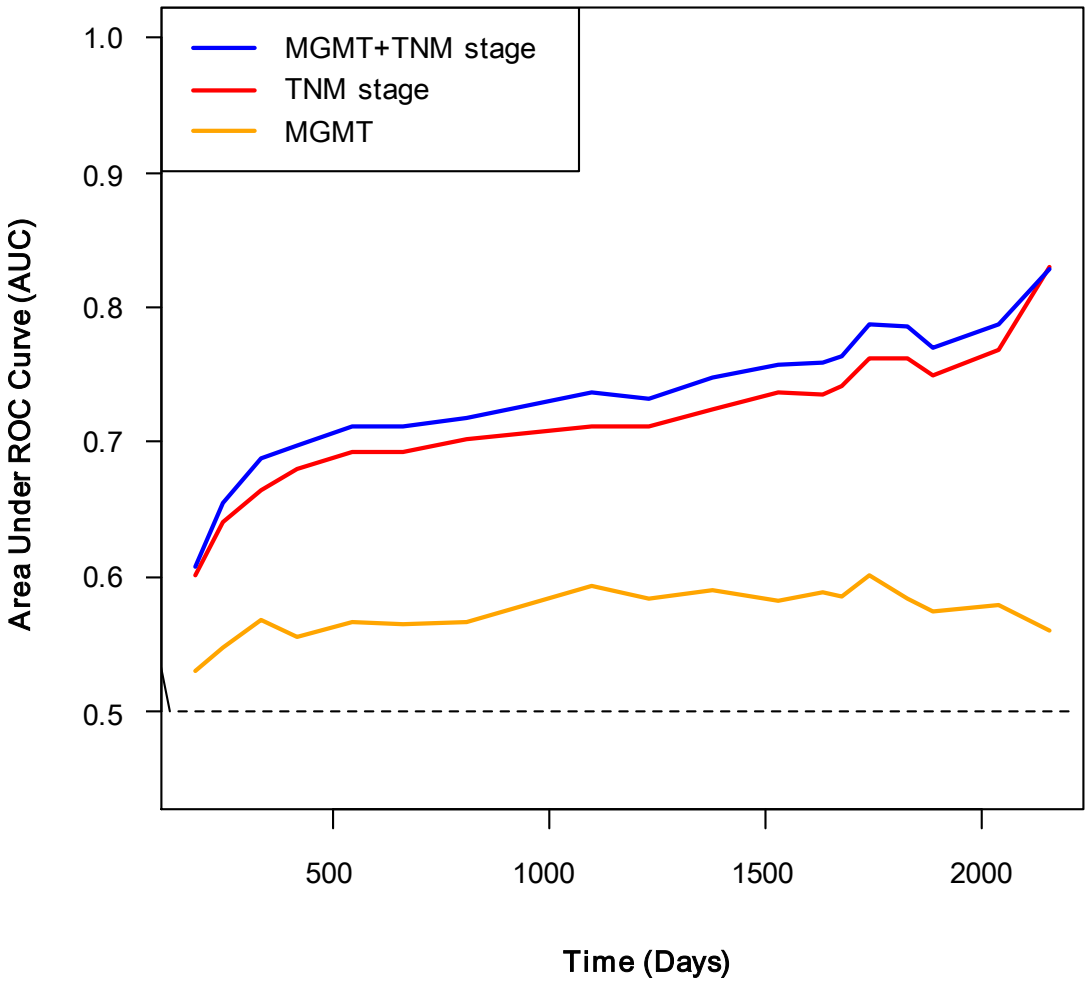
[b]Patients with adjuvant chemotherapy received at least one cycle of 5-fluoruracil based chemotherapy.

eTable 2. Tests of Proportional Hazards Assumption

Factors	Training set					Validation set			
	rho	**chi2**	**df**	**Prob>chi2**		**rho**	**chi2**	**df**	**Prob>chi2**
Age[a]	0.03197	0.08	1	0.78		-0.16139	3.71	1	0.054
Gender	-0.01011	0.01	1	0.92		-0.09636	1.21	1	0.27
Lauren classification	-0.08753	0.61	1	0.44		0.05462	0.37	1	0.54
Differentiation	0.01848	0.03	1	0.87		-0.09166	1.17	1	0.28
TNM stage[a]	0.13505	1.51	1	0.22		0.09836	1.30	1	0.26
MGMT expression	-0.05023	0.21	1	0.64		-0.13206	2.36	1	0.12
Global test		3.10	6	0.80			8.31	6	0.22

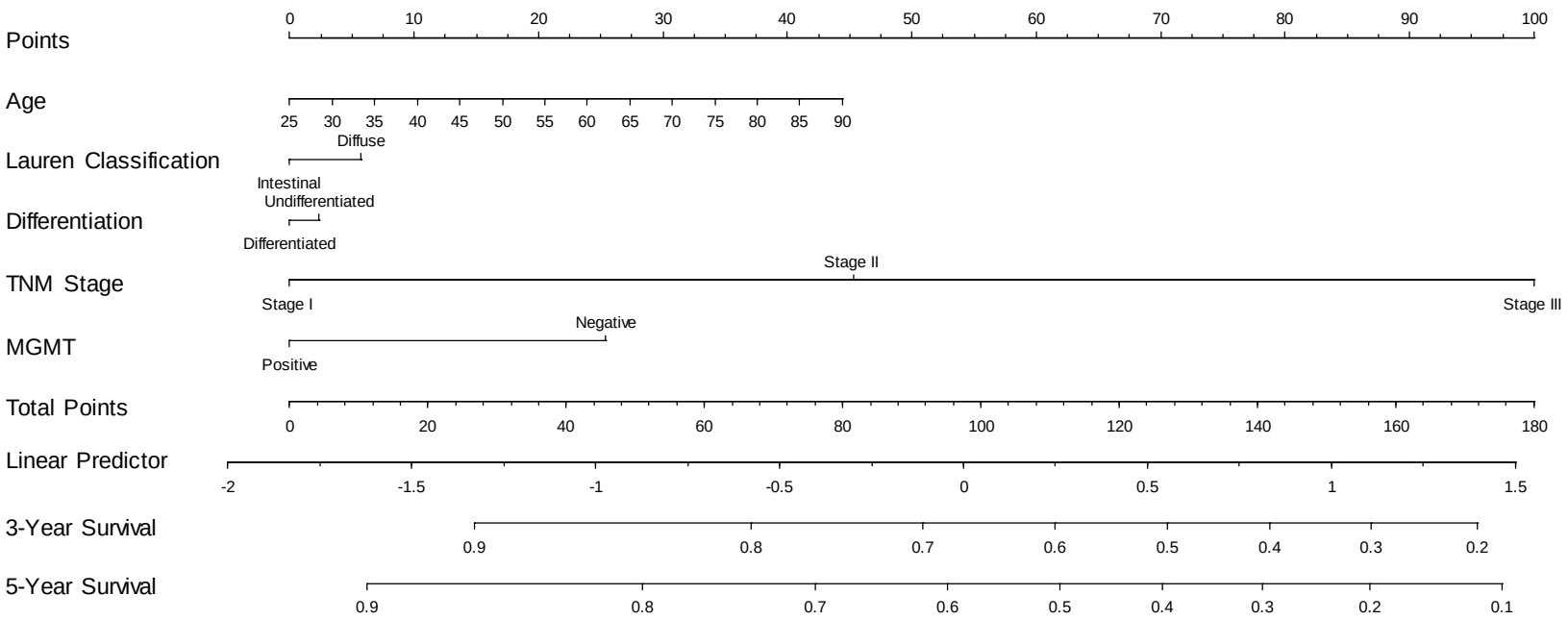
Abbreviations: MGMT= O^6-methylguanine-DNA methyltransferase; TNM = Tumor-Node-Metastasis.
[a]Modeled as a continuous variable

eFigure 1. Time-Dependent AUC Analysis to Show Prognostic Power of MGMT Expression and TNM Stage



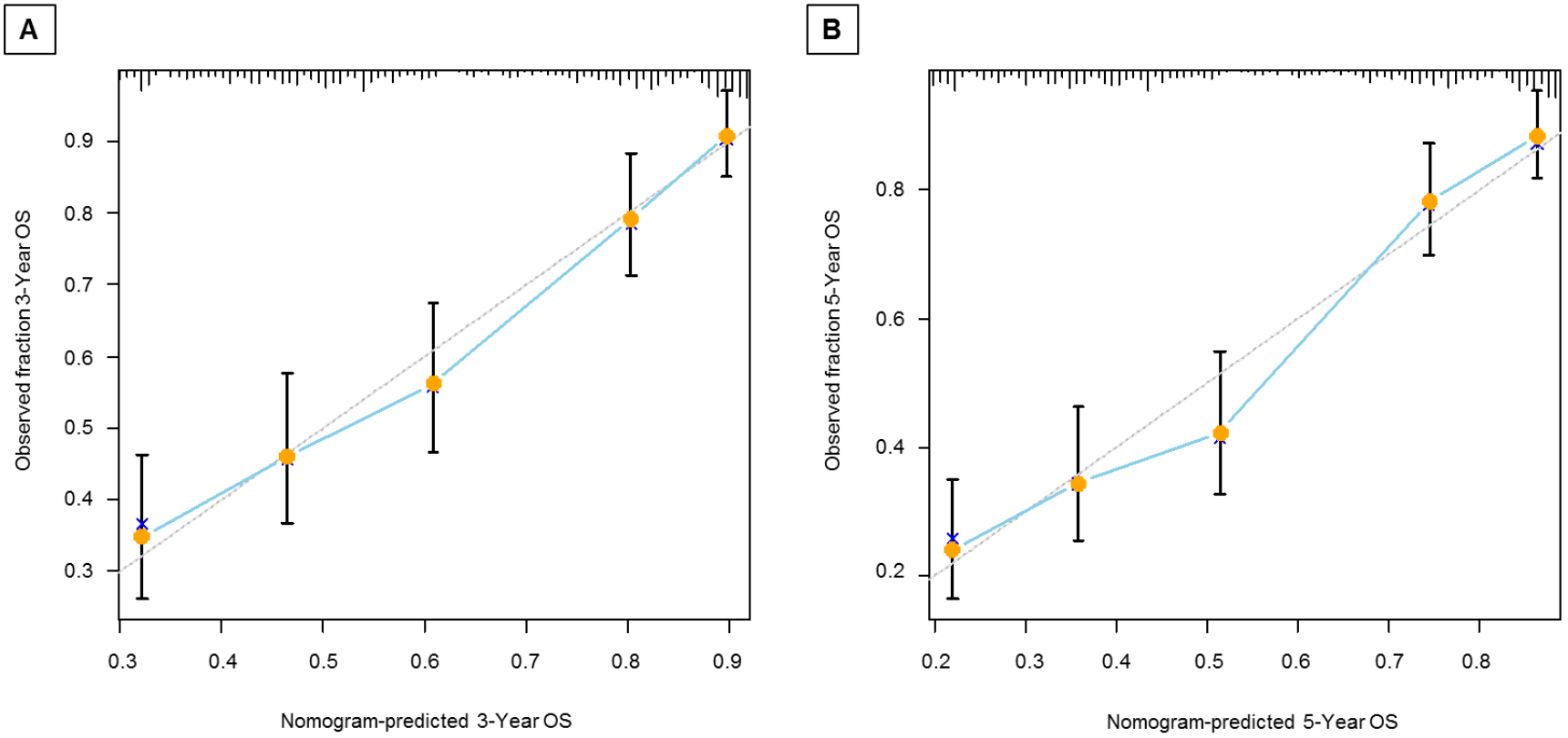
Time-dependent area under the ROC curve (AUC) analysis shows the prognostic capacity of MGMT and TNM stage. Improvement in prognostic power is also observed after the incorporation of MGMT expression into the current TNM staging system. Horizontal axis represents survival time (Days). Vertical axis represents area under the ROC curve (AUC).

eFigure 2. Gastric Cancer Survival Nomogram



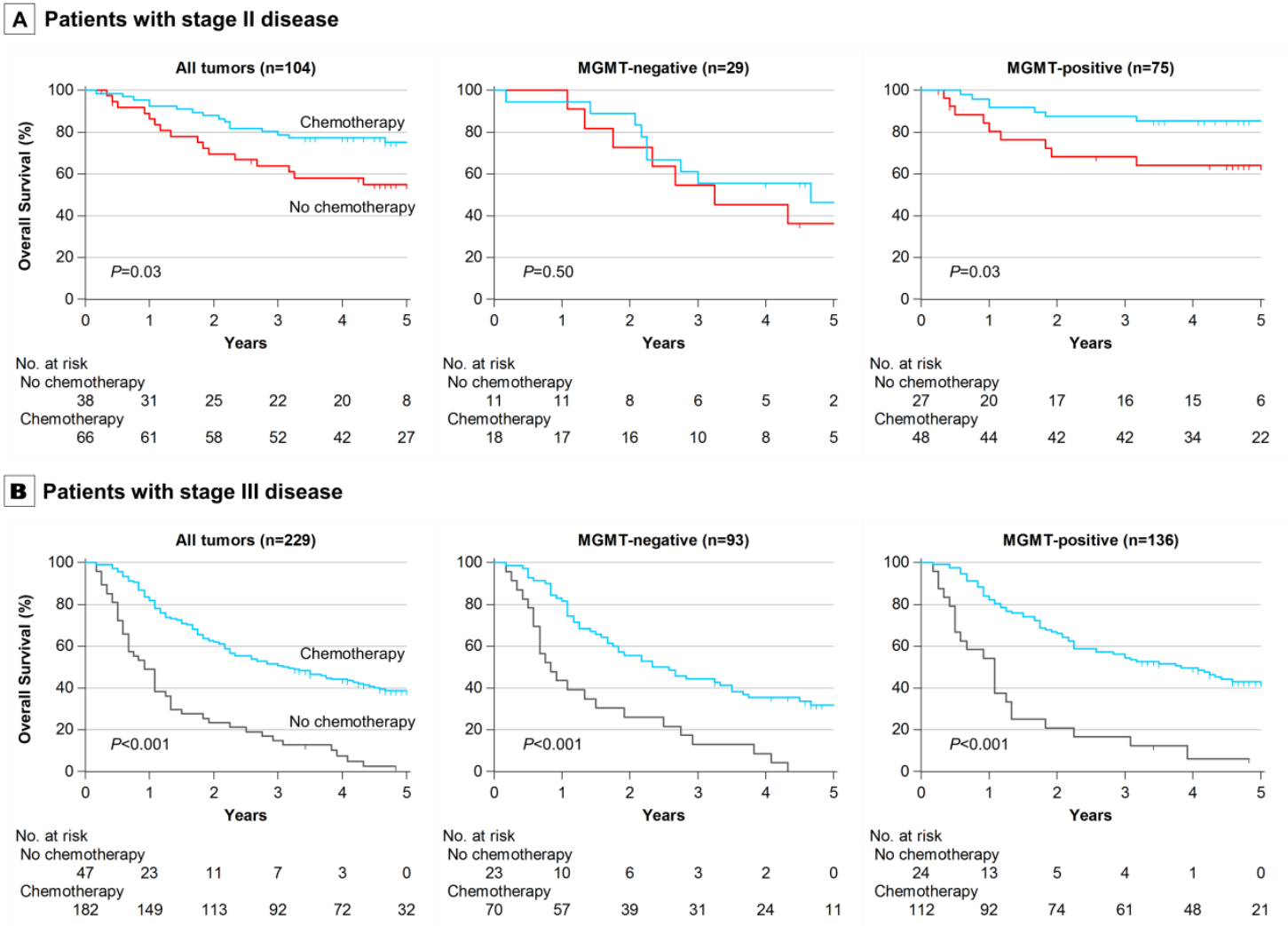
This nomogram provides a prognostic model to calculate 3-year and 5-year probability of postoperative overall survival on the basis of patient's combination of age, Lauren classification, tumor differentiation, TNM stage and MGMT expression. To use the nomogram, locate the patient's age, and draw a line straight up to the Points axis to obtain the score for age. Repeat for the other four covariates (Lauren Classification, Differentiation, TNM Stage and MGMT). Add the scores for each covariate together and locate the total score on the Total Points axis. Draw a line straight down to the 3-Year or 5-Year Survival axis to obtain the probability of overall survival (OS).

eFigure 3. Calibration Plots for the Survival Nomogram



The calibration curve for predicting gastric cancer patient overall survival (OS) at (A) 3 years and (B) 5 years. Nomogram-predicted probability of OS is plotted on the horizontal axis; actual observed OS is plotted on the vertical axis.

eFigure 4. Association Between MGMT Expression and Benefit From Fluorouracil-Based Adjuvant Chemotherapy



(A) In TNM stage II disease, MGMT-positive patients (n=75) could significantly benefit from adjuvant chemotherapy (*P*=0.03), whereas no significant difference was observed with respect to OS in MGMT-negative patients (n=29) when they were given adjuvant chemotherapy (n=18) or not (n=11) (*P*=0.50). (B) In TNM stage III disease, either MGMT-negative or MGMT-positive patients could benefit from adjuvant chemotherapy (*P*<0.001 and *P*<0.001, respectively).