Appendix

Table of contents:

Appendix A1 - Eligibility criteria and procedures

Development cohort: the LAP07 trial

Eligibility criteria

Main eligibility criteria were: histologically proven adenocarcinoma of the pancreas, de novo locally advanced unresectable tumour (stage III according to the UICC 2002 classification), measurable or evaluable disease (RECIST 1.0 criteria), no prior abdominal radiotherapy nor chemotherapy for any reason, performance status 0-2 (according to the WHO classification), and adequate biological tests (blood, liver, and kidney).
Main exclusion criteria were: stage IA to IIB or stage IV cancer (according to the TNM UICC 2002 classification), ampullary and periampullary carcinomas, prior chemotherapy (for any reason), abdominal radiotherapy, or treatment with an anti-EGFR, and allergy to any erlotinib ingredients.

Treatment and procedures

At enrolment, the first randomisation to gemcitabine versus gemcitabine plus erlotinib was performed using a minimization procedure with stratification according to centre and WHO performance status (0-1 versus 2). For patients whose tumour was controlled after 4 months of induction chemotherapy a second randomisation to chemoradiotherapy versus chemotherapy continuation was stratified by centre and the treatment given at the first randomisation.
The study was approved by the Comité de Protection des Personnes (CPP) de l'Île de France (French advisory committee for the Protection of Subjects in Biomedical Research, Ile de France) and conducted in agreement with article L.1123-6 of the Public Health Code. All enrolled patients provided written informed consent.

External validation cohort: consecutive patients treated at University Hospital of Besancon

Eligibility criteria

Main eligibility criteria were: histologically proven adenocarcinoma of the pancreas, de novo locally advanced unresectable tumour (stage III according to the UICC 2002 classification), measurable or evaluable disease (RECIST 1.0 criteria), at least one administration of chemotherapy-based treatment between January 1, 2003 and December 31, 2013.
Main exclusion criteria were: stage IA to IIB or stage IV cancer according to the TNM classification (UICC 2002), ampullary and periampullary carcinomas, absence of chemotherapy-based treatment between January 1, 2003 and December 31, 2013.

Treatment and procedures

All patients enrolled in the external validation cohort (n=106) were first treated with gemcitabine (51; 48%), gemcitabine and oxaliplatin (15; 14%), gemcitabine plus other drug except oxaliplatin (8; 8%), and FOLFOX with/without irinotecan (32; 30%)

This prospective population based cohort was constructed based on retrospectively collected data including baseline characteristics and outcomes information. The database was declared at the National French Commission for bioinformatics data and patient liberty (CNIL).

Appendix A2 – Statistical analyses interpretation

Discrimination

The C-index estimates the proportion of all pairwise patient combinations from the sample data whose survival time can be ordered such that the patient with the highest predicted survival is the one who actually survived longer (discrimination). The C-index ($0 \leq C \leq 1$) is a probability of concordance between predicted and observed survival, with C-index = 0.5 for random predictions and C-index = 1 for a perfectly discriminating model.

Calibration

Calibration refers to the ability to provide unbiased survival predictions in groups of similar patients. A prediction model is considered “well-calibrated” if the difference between predictions and observations in all groups of similar patients is close to 0 (perfect calibration). Any large deviation ($P < 0.1$) indicates a lack of calibration.

Bootstrapping

Bootstrapping is the preferred simulation technique that was first described by Bradley Efron. The original dataset is a random sample of patients being representative of a general population. Bootstrapping means generating a large number of datasets, each of which with the same sample size as the original one, by resampling with replacement (i.e., a previously selected patient may be selected again).

Internal validation

Internal validation is useful to obtain an honest estimate of the model performance for patients that are similar to those in the development sample and to indicate an upper limit to the expected performance in other settings. The bootstrap approach is the preferred technique to assess internal validity.

External validation

External validation may show different results from internal validation, since many aspects may be different between settings, including selection of patients, definition of variables, and diagnostic or therapeutic procedures. The strength of the evidence for the score validity is usually considered to be stronger with a fully external validation (other investigators, centres, etc.).

Appendix Table A1: (A) Final multivariate model adjusted for R1 treatment (*N* = 358), (B) Final multivariate model adjusted for grading and systolic blood pressure (*N* = 216), (C) Final multivariate model multiple imputation analysis.

A

	Nr of patients	Nr of deaths	HR	95% CI	*P*
Age at diagnosis, years	358	307	1.01	1.00 to 1.02	0.054
Pain					
No	155	130	1	-	
Yes	203	177	1.29	1.02 to 1.63	0.033
Albumin, g/L	358	307	0.96	0.94 to 0.98	0.001
Tumour size, mm	358	307	1.01	1.00 to 1.01	0.027
CA 19-9, UI/mL (log-value)	358	307	1.17	1.04 to 1.31	0.007
R1 treatment					
Gemcitabine	179	153	1	-	
Gemcitabine* + *Erlotinib	179	154	1.11	0.88 to 1.39	0.366

B

	Nr of patients	Nr of deaths	HR	95% CI	*P*
Age at diagnosis, years	216	187	1.02	1.01 to 1.04	0.008
Pain *No*	87	73	1		
Yes	129	114	1.44	1.05 to 1.96	0.022
Albumin, g/L	216	187	0.97	0.94 to 0.99	0.022
Tumour size, mm	216	187	1.01	1.00 to 1.02	0.066
CA 19-9, UI/mL (log-value)	216	187	1.03	0.89 to 1.20	0.703
Systolic blood pressure, mmHg	216	187	1.01	1.00 to 1.02	0.004
Well differentiated	42	33	1		
Moderately differentiated	33	27	1.53	0.91 to 2.58	
Poorly differentiated	22	21	1.78	1.01 to 3.14	
Not assessed	119	106	1.57	1.05 to 2.35	0.114

Abbreviations: CI: denotes confidence interval, HR: Hazard Ratio

C

	Complete-subject analysis (*N* = 358)			Multiple imputation analysis (MCMC, *N* = 1000 imputed dataset)		
Parameter	**ß**	**SE**	***P***	**ß**	**SE**	***P***
Age at diagnosis, years	0.0125	0.0063	0.0478	0.01217	0.00560	0.0298
Pain	0.2562	0.1193	0.0317	0.23995	0.11055	0.0300
Albumin, g/L	-0.0382	0.0111	0.0006	-0.03911	0.01085	0.0003
Tumour size, mm	0.0080	0.0035	0.0214	0.00810	0.00332	0.0148
CA 19-9, UI/mL (log-value)	0.1587	0.0573	0.0056	0.11556	0.05268	0.0283

Appendix A3 - General theoretical aspects

Risk estimation by a Cox model

The survival estimate for the patient j at time t based on the Cox model is computed as follows:

General formula:

$$S(t, X_j) = S_0(t)^{exp(\sum_{i=1}^{p} \beta_i X_{ij} - \sum_{i=1}^{p} \beta_i \overline{X_\iota})} S(t, X_j) = S_0(t)^{exp\left(\sum_{i=1}^{p} \beta_i (X_{ij} - \overline{X_\iota})\right)}$$

where:

- ßi is the estimated regression coefficient i=1..p
- S0(t) is the baseline survival at time t
- Xij the value of the ith risk factor for the patient j
- $\overline{X_\iota}$ the mean of value of the risk factor i in the population (to compute if the risk factor is quantitative)
- And p denotes the number of risk factors.

Nomogram development

Coefficients of the final Cox regression model were used to generate a nomogram allowing individual median survival and survival probability predictions at different time points (6, 12, 24, and 48 months).

Survival probability estimation at time of interest

The Cox regression linear predictor function (LPj) compared with the average risk profile was obtained by summing up the products between the characteristic i of patient j (Xij) less the average value of the characteristic (if quantitative) and corresponding Cox coefficient (βi):

$$\mathrm{LP_j} = \sum_{i=1}^{p} \beta_i \times \left(X_{ij} - \overline{X_\iota}\right)$$

For example, the 6-month survival probability for a patient j is given by the following formula:

$$\mathrm{S(6, X_j)} = \mathrm{S_0(6)^{\exp\left(\sum_{i=1}^{p} \beta_i (X_{ij} - \overline{X_\iota})\right)}} = \mathrm{S_0(t)^{\exp(LP_j)}}$$

where, S0(6) is the basis risk at 6 months.

Median survival estimation

Median overall survival by definition is the time, τ, such that S(τ) = 0.5. However, in practice, it is defined as the smallest time such that observed S(τ) ≤ 0.5. The median is more appropriate for censored survival data than the mean.
With a mathematical formulation:

$$Median\ OS_j = \ min\left(t \ / \ S(t, X_j) \leq \ \frac{1}{2}\right)$$

$$S(t, X_j) = \ S_0(t)^{exp(\mathrm{LP_j})} = \frac{1}{2}$$

$$\Leftrightarrow ln\left(\ S_0(t)^{exp(\mathrm{LP_j})}\right) \ = ln\left(\frac{1}{2}\right)$$

$$\Leftrightarrow \ exp(LP_j) ln(\ S_0(t)\ ) \ = ln\left(\frac{1}{2}\right)$$

$$\Leftrightarrow \ ln(\ S_0(t)\ ) \ = ln\left(\frac{1}{2}\right) exp(-LP_j)$$

$$\Leftrightarrow t \ / \ S_0(t) \ = \exp\left(ln\left(\frac{1}{2}\right) exp(-LP_j)\right)$$

$$\min\ (t) \ / \ S_0(t) \ = \exp\left(ln\left(\frac{1}{2}\right) exp(-LP_j)\right)$$

Survival estimation confidence interval

The confidence limits for individual survival estimations need firstly to derived confidence limits around the Cox regression linear predictor function (LPj).

The 97.5th quantile of the standard normal distribution is 1.96.

Indeed, with

the $\left(\ 100 - \frac{\alpha}{2}\right)$th quantile of the standard normal distribution is equal to a

$$(\ 1 - \alpha\ )\%\mathrm{CI}\ \mathrm{LP_j}$$
$$= \left[\mathrm{Lower\ LP_j};\ \mathrm{Upper\ LP_j}\right]$$
$$= \left[\mathrm{LP_j} - \ \mathrm{a}\ \times \mathrm{Standard\ Error}\left(\mathrm{LP_j}\right);\ \mathrm{LP_j} + \ \mathrm{a}\ \times \mathrm{Standard\ Error}(\mathrm{LP_j})\right]$$

And then for example a time t,

$$(\ 1 - \alpha\ )\%\mathrm{CI}\ \mathrm{S}\left(\mathrm{t}, \mathrm{X_j}\right) = (\ 1 - \alpha\ )\%\mathrm{CI}\ \mathrm{S_0(t)}^{\exp\left(\mathrm{LP_j}\right)}$$
$$= \left[\mathrm{S_0(t)}^{\exp\left(\mathrm{Upper\ LP_j}\right)}\ ;\ \mathrm{S_0(t)}^{\exp\left(\mathrm{Lower\ LP_j}\right)}\right]$$

So For a patient j we need to calculate the standard error of its Cox regression linear predictor LPj.

If we consider

$X_j = \begin{pmatrix} x_{1,j} \\ x_{2,j} \\ \vdots \\ x_{p-1,j} \\ x_{p,j} \end{pmatrix}$ the vector for the risk factor values for the patient j

$\overline{X} = \begin{pmatrix} \overline{X}_1 \\ \overline{X}_2 \\ \vdots \\ \overline{X}_{p-1} \\ \overline{X}_p \end{pmatrix}$ the vector for the mean of the risk factor observed in the patients involved in the cox multivariate analysis

$VCov(X) = \begin{pmatrix} Var(X_1) & Cov(X_1, X_2) & \cdots & Cov(X_1, X_p) \\ Cov(X_2, X_1) & \ddots & \ldots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ Cov(X_p, X_1) & \cdots & \ldots & Var(X_p) \end{pmatrix}$ the covariance matrix (issued from the model)

Then

$\text{Standard Error}(LP_j) = t(X_j - \overline{X}) \times VCov(X) \times (X_j - \overline{X})$

Risk factors and attributed points

For each factor, the maximum score assigned to each variable presented in Table 3 are provided by the following formula:

For a risk factor i the maximal points equal to:

$$\text{Points\_max}_i = 100 \times \frac{\beta_i |\max_i - \min_i|}{\beta_j |\max_j - \min_j|} = 100 \times \frac{\text{Absolute range } \beta_i \text{ value}}{\text{Absolute range } \beta_j \text{ value}}$$

where the factor j is that with the maximum absolute range β value and max and min are the limits of the factor variation with a sorting value less to higher risk with the outcome.

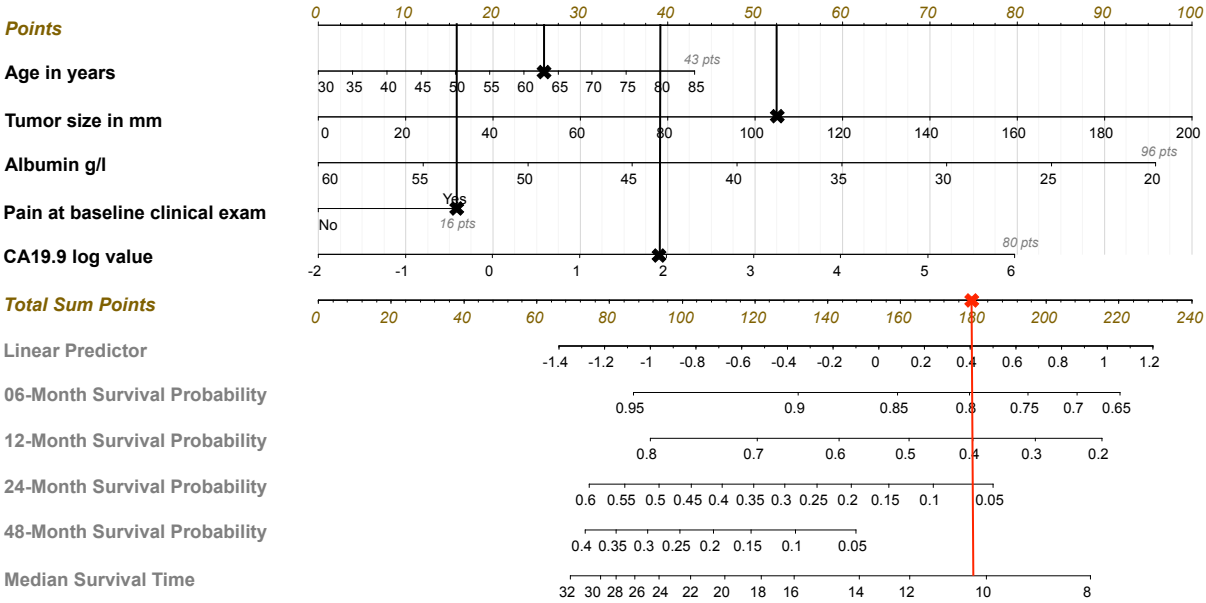
Then, the points for a patient j with the factor i equal to b are deduced as following:

$$Points_{ij} = |b - min_i| \times \frac{Score\_max_i}{|max_j - min_j|}$$

The total points score for a patient j is equal to:

$$\text{Total Points}_j = \sum_i \text{Points}_{ij}$$

Nomogram Illustration



Construction of the PROLAP prognostic score

Derived from the multivariate final Cox model:

$$\text{Raw prognostic score}_{\text{Cox model}} = \begin{matrix} \text{Age in years} \times \alpha \\ \text{Pain } \binom{0}{1} \times \beta \\ \text{Albumin in g/L} \times \gamma \\ \text{Tumor size in mm} \times \delta \\ \text{CA19.9 Log value} \times \varepsilon \end{matrix}$$

$\alpha, \beta, \gamma, \delta$ and ε : Cox-model beta coefficient for the corresponding parameters.

$$\text{Raw prognostic score}_{\text{Cox model}} \in [\min\_score - \max\_score]$$
$$: theorical\ range\ with\ the\ best\ and\ the\ worst\ profile\ for\ each\ risk\ factors$$

$$\text{Normalized prognostic score}_{\text{Cox model}} = \frac{(5-0)\times(\text{Raw Prognostic score}_{\text{Cox model}} - \min\_score)}{\max\_score - \min\_score} + 0$$

$$\text{Normalized prognostic score}_{\text{Cox model}} \in [0-5]$$

Derived from the nomogram:

If the attributed points for each risk factor in the nomogram are considered, a raw prognostic score derived from the nomogram can be calculated as follows:

$$\text{Raw prognostic score}_{nomogram} = \begin{matrix} (\text{Age in years} - \min) \times \frac{\max points\_age}{\max\_age - min\_age} \\ \text{Pain } \binom{0}{1} \times \text{maxpoints}\_age \\ \left|\text{Albumin in}\frac{\text{g}}{\text{L}} - \min\_albumin\right| \times \frac{\max points\_albumin}{|\max\_albumin - \min\_albumin|} \\ (\text{Tumor size in mm} - \min\_tumorsize) \times \frac{max\ points\_tumorsize}{max\_tumorsize - min\_tumorsize} \\ |\text{CA19.9 Log value} - \min\_CA19.9| \times \frac{\max points\_CA19.9}{|\max\_CA19.9 - \min\_CA19.9|} \end{matrix}$$

$$\text{Raw prognostic score}_{nomogram} \in [\min\_score\ -max\_score]$$

$$\text{Normalized prognostic score}_{nomogram} = \frac{(5-0)\times\left(\text{Raw Prognostic score}_{nomogram} - \min\_score\right)}{\max\_score - \min\_score} + 0$$

$$\text{Normalized prognostic score}_{nomogram} \in [0-5]$$

$$\text{Normalized prognostic score}_{nomogram} = \text{Normalized prognostic score}_{\text{Cox model}}$$

Determination of cut-off values in total sum of points derived from the nomogram in order to determine groups issued from the normalized prognostic score of the model:

We previously observed that:

$$\text{Normalized Prognostic score}_{nomogram} = \text{Normalized Prognostic score}_{\text{Cox model}}$$

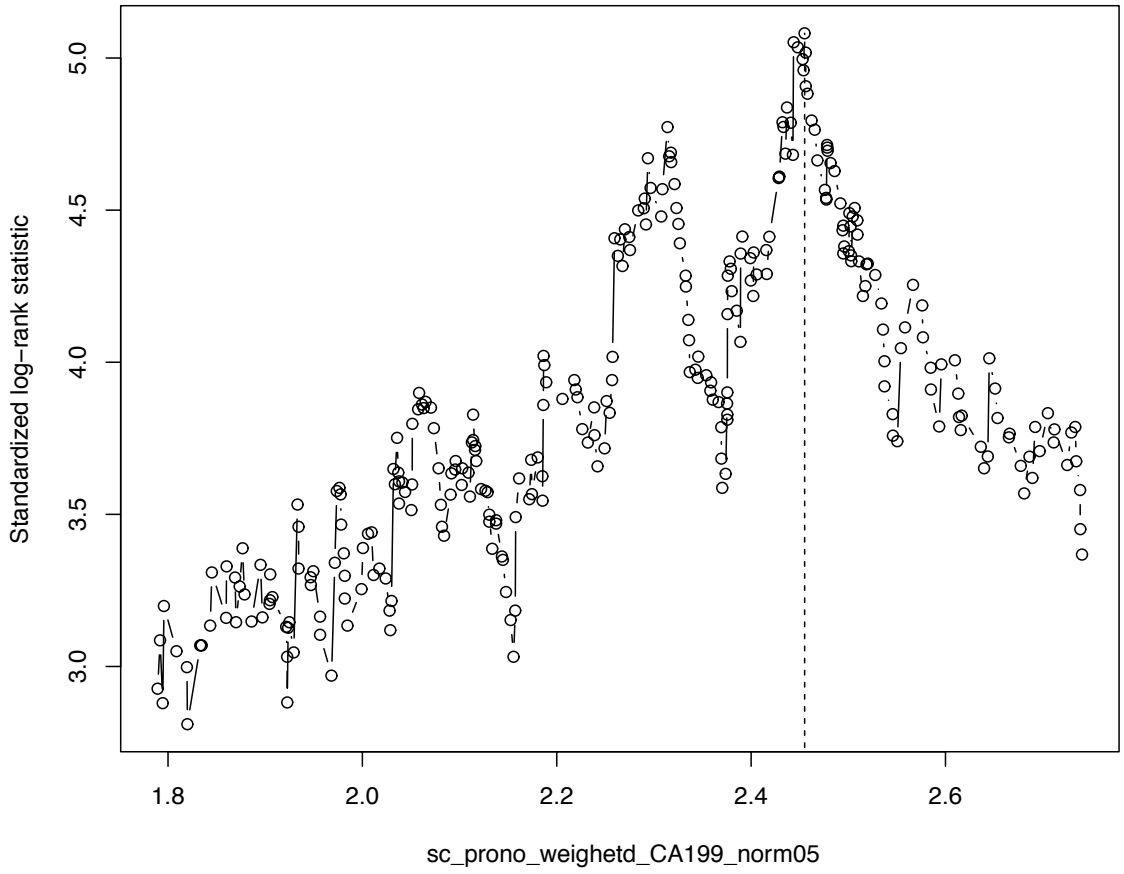
$$\frac{(5-0)\times\left(\text{Raw Prognostic score}_{nomogram} - \min\_score\right)}{\max\_score} + 0 = \text{Normalized Prognostic score}_{\text{Cox model}}$$

$$\text{Normalized Prognostic score}_{\text{Cox model}} = 2.29 \rightarrow \text{Raw Prognostic score}_{nomogram} = 155.3$$

Given these calculations, the two risk groups of patients can be characterized as following:

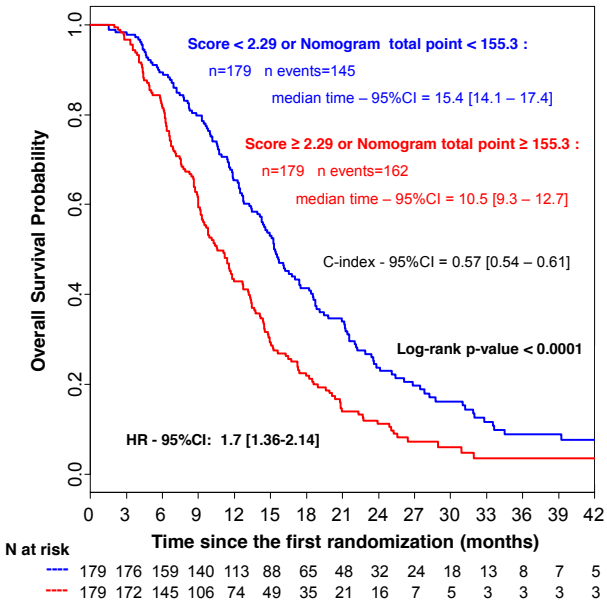
- Low-risk group: a score < 2.29 or a total number of points from the nomogram < 155.3,
- High-risk group: a score ≥ 2.29 or a total number of points by the nomogram ≥ 155.3.

Appendix Figure A1. An optimal cut point determined by Hothorn & Lausen method

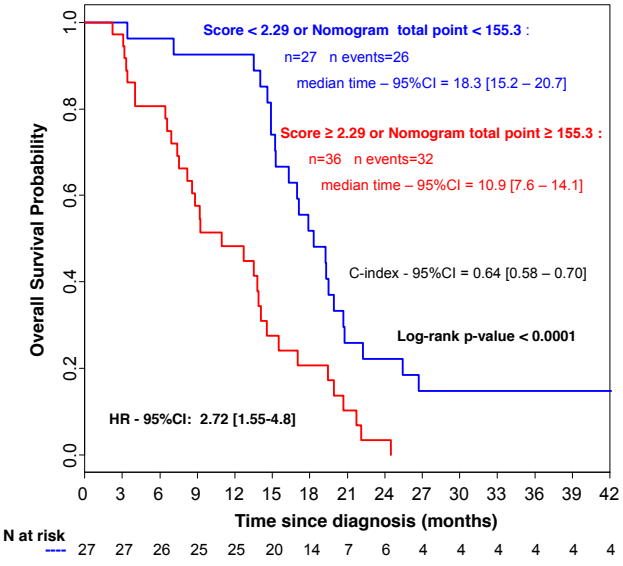


Appendix Figure A2: Kaplan-Meier curves for overall survival in (A,C) the development set cohort and (B,D) the external validation set cohort according to the prognostic score group using the median and tertile approach

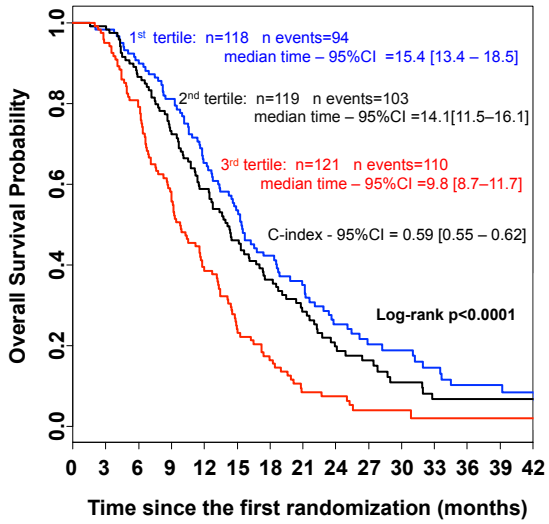
A



B



C



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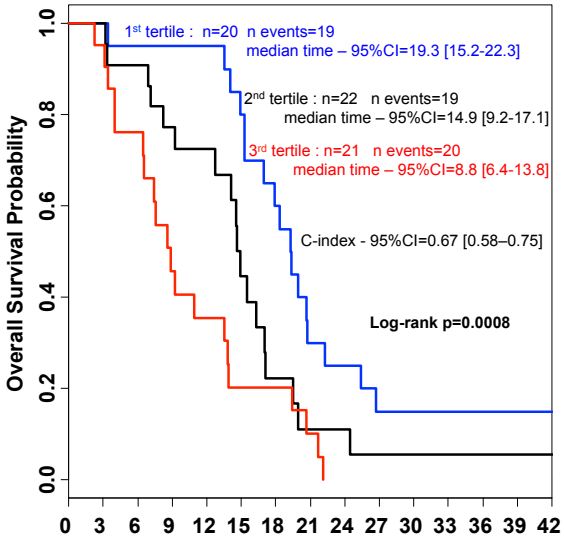

Appendix Table A1. Patient characteristics in (A) two risk and (B) three prognostic risk groups

A

	Prognostic risk group		
	Low [0,2.455] *N* = 242	**High [2.455,5] *N* = 116**	**Global *P***
Age at diagnosis, years*	61.4 ± 9.5	68.1 ± 7.8	< 0.0001
Pain			
No	126 (52%)	29 (25%)	
Yes	116 (48%)	87 (75%)	< 0.0001
Albumin, g/L*	39.4 ± 5.1	34.7 ± 5.3	< 0.0001
Tumour size, mm*	40.4 ± 13.9	52.4 ± 22.9	< 0.0001
CA 19.9, UI (log-value)*	2.0 ± 1.0	3.1 ± 0.9	< 0.0001

* Plus–minus values are means ± standard deviation

B

	Prognostic risk group			
	Low [0,2.291] *N* = 178	**Intermediate [2.291,2.720] *N* = 137**	**High [2.720,5] *N* = 43**	**Global *P***
Age at diagnosis, years*	60.2 ± 9.3	66.4 ± 9.3	68.6 ± 7.6	< 0.0001
Pain				
No	101 (57%)	45 (33%)	9 (21%)	
Yes	77 (43%)	92 (67%)	34 (79%)	< 0.0001
Albumin, g/L*	40.2 ± 5.0	36.7 ± 4.8	32.1 ± 5.3	< 0.0001
Tumour size, mm*	37.7 ± 12.0	47.4 ± 16.3	61.7 ± 28.4	< 0.0001
CA 19.9, UI (log-value)*	1.8 ± 1.0	2.7 ± 0.9	3.3 ± 0.9	< 0.0001

* Plus–minus values are means ± standard deviation

Appendix A4 Predictive value of treatments on OS according to the risk groups

Two risk group approach

There were no differential effects on OS for the gemcitabine-erlotinib combination across the two risk groups (high: log-rank *P* = 0.2209; low: log-rank *P* = 0.9579). A total of 162 (67%) low-risk and 65 (56%) high-risk patients reached the second randomization (*P* = 0.047). Similarly, there was no significant OS difference in favour of chemoradiotherapy over chemotherapy across the two risk groups (low: log-rank *P* = 0.5963; high: *P* = 0.8334).

Three risk group approach

There were no differential effects on OS for the gemcitabine-erlotinib combination across the three risk groups (high: log-rank *P* = 0.1420; intermediate: log-rank *P* = 0.9376; low: log-rank *P* = 0.2601). A total of 121 (68%) low-risk, 85 (62%) intermediate risk, and 19 (44%) high-risk patients reached the second randomization (*P* = 0.0027). Similarly, there was no significant OS difference in favour of chemoradiotherapy over chemotherapy across the three risk groups (high: log-rank *P* = 0.8646; intermediate: log-rank *P* = 0.4082; low: log-rank *P* = 0.4924).

Appendix Table A2. Baseline characteristics of the external validation set cohort according to the eligibility status for staging system

		Patient eligible for staging system (*N* = 63)	Patient not eligible for staging system (*N* = 43)	*P*
Age, years*		67.1 ± 10.6	67.1 ± 9.7	0.9790
Gender, *N* (%)	*Male*	33 (52%)	24 (56%)	
	Female	30 (48%)	19 (44%)	0.7278
Localization, *N* (%)	*Head/Head and Body*	42 (69%)	29 (71%)	
	Other (body and/or tail)	19 (31%)	12 (29%)	0.8397
	Unknown	2	2	
Grading, *N* (%)	*Well differentiated*	4 (17%)	5 (45%)	
	Moderately differentiated	14 (61%)	3 (27%)	
	Poorly differentiated	5 (22%)	3 (27%)	0.1336
	Missing	40	32	
PS (WHO), *N* (%)	*0*	19 (30%)	12 (29%)	
	1	33 (52%)	26 (62%)	
	2	10 (16%)	4 (10%)	
	3	1 (2%)	0 (0%)	0.7048
	Missing	0	1	
RECIST tumour size (mm)*		38.0 ± 14.9	38.9 ± 14.4	0.7727
	Missing	0	9	
RECIST tumour size (mm), *N* (%)	*<30*	16 (25%)	6 (18%)	
	30-50	34 (54%)	22 (65%)	
	≥50	13 (21%)	6 (18%)	0.5694
	Missing	0	9	
Median overall survival time in months 95%CI †		14.9 (13.5 to 17.1)	14.9 (11.1 to 21.3)	0.2465

* Plus–minus values are means ± standard deviation

† Compared with log-rank test

Abbreviations: PS, performance status, WHO: World Health Organization.