

## Supplementary data

Table of contents:

Supplementary methods (Page 2)

Supplementary table (Page 3)

Supplementary references (Page 4)

## **SUPPLEMENTARY METHODS**

### **Ang-2 plasma sample measurement**

As previously described (1), blood samples were drawn at baseline and immediately processed for plasma and serum freezing at  $-80^{\circ}\text{C}$ . Samples frozen more than 4 hours following venous blood collection were excluded from the analysis. Enzyme-linked immunosorbent assays (ELISA) were used to measure Ang-2 in serum samples (R&D systems and Diaclone) according to the manufacturer's instructions. Each sample was analysed in duplicate.

### **Statistical analysis 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  $C=0.5$  for random predictions and  $C=1$  for a perfectly discriminating model.

#### Calibration

Calibration refers to the ability to provide unbiased survival predictions in groups of similar patients. A prediction model was considered "well calibrated" if the difference between predictions and observations in all groups of similar patients was close to 0 (perfect calibration). Any large deviation ( $p < 0.1$ ) indicated lack of calibration.

#### Bootstrapping

Bootstrapping is the preferred simulation technique and was first described by Bradley Efron.(2) The idea is that the original dataset is a random sample of patients 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 (*ie.* an already selected patient may be selected again).

#### Risk reclassification

Continuous net reclassification is the sum of the net percentages of persons with and without the event of interest correctly assigned a different predicted risk; this statistic cannot be interpreted as a percentage. Theoretical range is  $-2$  to  $2$ . cNRI was originally proposed to overcome the problem of selecting categories in applications where they do not naturally exist.(3)

SUPPLEMENTARY TABLE

	Number of patients	Number of deaths	Univariate unstratified analysis			Univariate stratified analysis		
			HR	95%CI	P	HR	95%CI	P
<b>Age — years</b>								
<= 65	93	84	1	-	-	1	-	-
>65	84	78	1.243	[0.912; 1.694]	0.1689	1.172	[0.857; 1.603]	0.3192
<b>Sex</b>								
Male	74	65	1			1		
Female	103	97	1.310	[0.955; 1.798]	0.0941	1.157	[0.839; 1.59]	0.3732
<b>Performance status</b>								
<b>OMS</b>								
0	91	80	1			1		
>0	76	73	1.274	[0.927; 1.751]	0.1355	1.148	[0.833; 1.581]	0.3999
<b>Primary Tumor site</b>								
Colon	120	113	1			1		
Rectum	57	49	0.855	[0.610; 1.196]	0.3597	1.032	[0.732; 1.457]	0.8559
<b>Timing to metastasis</b>								
Metachronous	119	111	1			1		
Synchronous	54	47	1.458	[1.035; 2.054]	0.0308	1.260	[0.892; 1.780]	0.1899
<b>Metastases localisation</b>								
Liver alone	68	59	1			1		
Liver and other	98	95	1.816	[1.305; 2.527]		1.632	[1.170; 2.276]	
Other alone	11	8	0.686	[0.326; 1.442]	0.0003	1.037	[0.465; 2.314]	0.0140
<b>Surgery of the primary tumor</b>								
Yes	110	101	1			1		
No	65	59	1.433	[1.035; 1.984]	0.0304	1.676	[1.198; 2.347]	0.0026
<b>Leucocyte (x10<sup>6</sup>/mL)*</b>	137	122	1.120	[1.056; 1.188]	0.0002	1.108	[1.044; 1.176]	0.0008
<b>Lymphocyte (x10<sup>6</sup>/mL)*</b>	132	120	0.795	[0.589; 1.073]	0.1339	0.823	[0.610; 1.111]	0.2037
<b>LDH ‡</b>								
<=350 (ULN)	56	49	1			1		
>350 (ULN)	59	58	2.026	[1.373; 2.988]	0.0004	1.897	[1.279; 2.812]	0.0014
<b>Log_Ang-2 (pg/mL)</b>	177	162	1.911	[1.492; 2.448]	<0.0001	1.814	[1.387; 2.373]	<0.0001

Supplementary Table 1: Cox Univariate analyses for OS prediction with and without the stratified approach (sensitivity analysis)

Red characters represent significant results

## SUPPLEMENTARY REFERENCES

- 1- Kim S, Dobi E, Jary M, Monnien F, Curtit E, Nguyen T et al. Bifractionated CPT-11 with LV5FU2 infusion (FOLFIRI-3) in combination with bevacizumab: clinical outcomes in first-line metastatic colorectal cancers according to plasma angiopoietin-2 levels. *BMC Cancer* 2013; 13: 611.
- 2- Efron (1979) Bootstrap Methods: Another Look at the Jackknife. *Ann. Stat.* . Vol. 7. 1-26.
- 3- Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011; 30(1): 11-21.