**SUPPLEMENTAL MATERIAL**

**Backen et al.**

**Table S1 Spearman correlations (rho) between biomarkers**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Ang2** | **FGFb** | **HGF** | **VEGFA** | **VEGFC** | **GCSF** | **IL8** | **KGF** | **PLGF** | **VEGFR1** | **VEGFR2** | **Ang1** | **PDGFbb** | **Tie2** | **VEGFD** |
| **Ang2** | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **FGFb** | 0.719 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **HGF** | 0.722 | 0.691 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |  |
| **VEGFA** | 0.627 | 0.731 | 0.712 | 1.000 |  |  |  |  |  |  |  |  |  |  |  |
| **VEGFC** | 0.577 | 0.702 | 0.640 | 0.821 | 1.000 |  |  |  |  |  |  |  |  |  |  |
| **GCSF** | 0.458 | 0.502 | 0.400 | 0.281 | 0.244 | 1.000 |  |  |  |  |  |  |  |  |  |
| **IL8** | 0.553 | 0.654 | 0.526 | 0.496 | 0.462 | 0.717 | 1.000 |  |  |  |  |  |  |  |  |
| **KGF** | 0.511 | 0.538 | 0.478 | 0.358 | 0.306 | 0.870 | 0.687 | 1.000 |  |  |  |  |  |  |  |
| **PLGF** | 0.538 | 0.560 | 0.413 | 0.331 | 0.314 | 0.869 | 0.690 | 0.875 | 1.000 |  |  |  |  |  |  |
| **VEGFR1** | 0.424 | 0.432 | 0.344 | 0.249 | 0.253 | 0.903 | 0.645 | 0.863 | 0.924 | 1.000 |  |  |  |  |  |
| **VEGFR2** | 0.167 | 0.142 | 0.180 | 0.085 | 0.108 | 0.506 | 0.278 | 0.486 | 0.569 | 0.649 | 1.000 |  |  |  |  |
| **Ang1** | 0.014 | 0.237 | 0.248 | 0.455 | 0.401 | -0.148 | 0.046 | -0.134 | -0.158 | -0.167 | -0.137 | 1.000 |  |  |  |
| **PDGFbb** | 0.240 | 0.362 | 0.320 | 0.562 | 0.541 | -0.073 | 0.132 | -0.037 | -0.049 | -0.088 | -0.076 | 0.567 | 1.000 |  |  |
| **Tie 2** | 0.233 | 0.176 | 0.293 | 0.311 | 0.115 | 0.030 | 0.123 | 0.012 | 0.034 | 0.024 | 0.186 | 0.269 | 0.106 | 1.000 |  |
| **VEGFD** | 0.553 | 0.597 | 0.490 | 0.463 | 0.461 | 0.659 | 0.823 | 0.636 | 0.671 | 0.636 | 0.251 | 0.028 | 0.101 | 0.102 | 1.000 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

The baseline biomarker distributions are described in Table S2 and Figure S2, by treatment arm (Table S3) and whether or not surgical treatment impacted upon levels (Table S4).

**Table S2 Pre-chemotherapy/bevacizumab concentrations of individual angiogenesis associated factors**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biomarker** | **n** | **unit** | **mean** | **median** | **IQR** | **skewness** | **kurtosis** |
|  |  |  |  |  |  |  |  |
| **Ang1** | 91 | pg/ml | 4012 | 2978 | 1383-5053 | 2.78 | 13.84 |
| **Ang2** | 91 | pg/ml | 789 | 487 | 311-796 | 1.64 | 4.51 |
| **FGFb** | 91 | pg/ml | 152 | 27.2 | 10.7-102.4 | 4.03 | 18.18 |
| **GCSF** | 91 | pg/ml | 112.5 | 38.8 | 1.7-101.3 | 4.56 | 25.57 |
| **HGF** | 91 | pg/ml | 938 | 716 | 433-1158 | 1.64 | 5.36 |
| **IL8** | 91 | pg/ml | 100.7 | 48.5 | 15.8-146.8 | 1.31 | 3.77 |
| **KGF** | 91 | pg/ml | 54.6 | 17.8 | 7.4-51.2 | 5.67 | 38.43 |
| **PDGF-bb** | 91 | pg/ml | 171.7 | 109.9 | 53.9-108.3 | 2.65 | 11.07 |
| **PLGF** | 91 | pg/ml | 161.1 | 27.3 | 7.7-151.7 | 3.29 | 14.68 |
| **Tie2** | 91 | pg/ml | 20123 | 18823 | 15353-24148 | 0.80 | 3.96 |
| **VEGFA** | 91 | pg/ml | 270 | 177 | 104-265 | 3.45 | 16.11 |
| **VEGFC** | 91 | pg/ml | 2772 | 2045 | 1300-2912 | 2.82 | 12.55 |
| **VEGFD** | 91 | pg/ml | 4303 | 1467 | 498-4956 | 1.66 | 4.30 |
| **VEGFR1** | 91 | pg/ml | 590.1 | 97.4 | 37.2-466.4 | 5.39 | 33.33 |
| **VEGFR2** | 91 | pg/ml | 7563 | 6503 | 4725-10932 | 0.48 | 1.95 |
| **Ca125** | 86 | U/ml | 462 | 105 | 48-276 | 4.38 | 24.75 |
|  |  |  |  |  |  |  |  |

IQR: interquartile range

**Table S3 – comparisons between biomarker parameters by treatment arm**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Standard** | |  | **Bevacizumab** | |  |
| **Biomarker** | n | median |  | n | median | P value |
|  |  |  |  |  |  |  |
| **Ang1** | 44 | 3688 |  | 47 | 2569 | 0.0969 |
| **Ang2** | 44 | 487 |  | 47 | 499 | 0.7208 |
| **FGFb** | 44 | 24 |  | 47 | 30 | 0.8271 |
| **GCSF** | 44 | 42 |  | 47 | 33 | 0.6365 |
| **HGF** | 44 | 745 |  | 47 | 490 | 0.9747 |
| **IL8** | 44 | 73 |  | 47 | 47 | 0.8864 |
| **KGF** | 44 | 19 |  | 47 | 16 | 0.8551 |
| **PDGF-bb** | 44 | 123 |  | 47 | 94 | 0.1795 |
| **PLGF** | 44 | 29 |  | 47 | 17 | 0.4529 |
| **Tie2** | 44 | 19259 |  | 47 | 17925 | 0.2696 |
| **VEGFA** | 44 | 186 |  | 47 | 172 | 0.4387 |
| **VEGFC** | 44 | 2184 |  | 47 | 2008 | 0.8864 |
| **VEGFD** | 44 | 1319 |  | 47 | 1604 | 0.5621 |
| **VEGFR1** | 44 | 144 |  | 47 | 76 | 0.6252 |
| **VEGFR2** | 44 | 6387 |  | 47 | 6813 | 0.6738 |
| **Ca125** | 42 | 74 (39-217)\* |  | 44 | 163  (76.5-340.5)\* | 0.0177 |

\*Interquartile ranges in parentheses.

**Table S4 – comparisons between biomarker parameters by time since surgery**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Greater than 4 weeks** | |  | **Less than 4 weeks** | |  |
| Biomarker |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| Ang1 | 56 | 3.01 |  | 35 | 2.68 | 0.6774 |
| Ang2 | 56 | 497 |  | 35 | 477 | 0.2120 |
| FGFb | 56 | 32.9 |  | 35 | 20.2 | 0.1333 |
| GCSF | 56 | 42.4 |  | 35 | 37.7 | 0.5625 |
| HGF | 56 | 890 (492-1231) |  | 35 | 526 (425-980) | 0.0422 |
| IL8 | 56 | 75.8 |  | 35 | 34.1 | 0.2017 |
| KGF | 56 | 19.7 |  | 35 | 13.7 | 0.6833 |
| PDGF-bb | 56 | 121 |  | 35 | 88 | 0.1365 |
| PLGF | 56 | 22.5 |  | 35 | 31.9 | 0.9900 |
| Tie2 | 56 | 19300  (16652-25598) |  | 35 | 17104  (14298-20823) | 0.0196 |
| VEGFA | 56 | 194 |  | 35 | 145 | 0.1211 |
| VEGFC | 56 | 2148 |  | 35 | 2004 | 0.7504 |
| VEGFD | 56 | 1739 |  | 35 | 1110 | 0.9675 |
| VEGFR1 | 56 | 93.8 |  | 35 | 125.5 | 0.6159 |
| VEGFR2 | 56 | 6031 |  | 35 | 7092 | 0.3378 |
| Ca125 | 53 | 83 |  | 33 | 147 | 0.241 |
|  |  |  |  |  |  |  |

\*Interquartile ranges in parentheses.

**Table S5 – Validation of the identified biomarkers in the training dataset using bootstrap resampling technique**

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker** | **Prognostic Significance** | **Predictive Significance** | |
|  | **Relative frequency by bootstrap (%)** | **Relative frequency by bootstrap (continuous, %)** | **Relative frequency by bootstrap (binary, %)** |
| **Ang1** | 8.1 | **52.5** | 6.7 |
| **Tie2** | **56.3** | 8.0 | **53.3** |
| **Ang2** | 4.7 | 7.5 | 17.9 |
| **FGFb** | 6.9 | 11.3 | 6.4 |
| **GCSF** | 1.9 | 11.0 | 5.6 |
| **HGF** | 6.4 | 12.2 | 7.7 |
| **IL8** | 2.8 | 7.1 | 5.7 |
| **KGF** | 3.0 | 9.1 | 10.7 |
| **PDGFbb** | 1.7 | 16.7 | 47.1 |
| **PlGF** | 5.4 | 13.3 | 14 |
| **VEGFA** | 6.8 | 2.9 | 5.6 |
| **VEGFC** | 8.9 | 3.4 | 13.3 |
| **VEGFD** | 5.3 | 6.6 | 20.8 |
| **VEGFR1** | 2.4 | 7.5 | 10.5 |
| **VEGFR2** | 9.7 | 7.7 | 8.5 |

**Statistical Analysis of Data as Continuous Variables**

The statistical approach included screening for prognostic and predictive interactive biomarkers, exploration of candidate biomarkers for unbiased cut-offs and exploration of biologically plausible combinations of clinically relevant predictive biomarkers. To identify variables of prognostic importance, we modelled each biomarker as a continuous single covariate in a Cox's model for PFS. We assumed linearity in the biomarker-hazard relationship and examined for evidence of nonlinearity by plotting the martingale residuals from each marker-specific analysis. We additionally dichotomized the data for each biomarker at its median value, and examined for prognostic significance using Kaplan-Meier curves and compared high- versus low-value categories using log-rank tests. To identify variables of predictive importance with respect to treatment effect, we considered each biomarker and tested for a possible departure of the data from additivity in the effect of that biomarker and the effect of the treatment, within a Cox's model. Presence of this interaction was interpreted as implying that the effect of bevacizumab changes with the value of the biomarker, and is consequently evidence in favor of the biomarker being used for therapeutic decision-making.

We explored candidate biomarkers for unbiased cut-offs, avoiding data-driven determination of ‘optimal’ cut-off points. For pragmatic reasons (both clinical and size of dataset), we a priori dichotomized variables at their respective medians, and examined for deviation in results obtained from Kaplan-Meier curves versus those for the biomarker as a continuous variable in the Cox’s Model. In general, we found no substantial deviation and worked with median cut-off dichotomized data in the main models. As additional tests of these assumptions, we investigated for interactions between treatment and continuous covariates using multivariable fractional polynomials interaction (MFPI) models, which allows determination of an unbiased cut-off point while avoiding restricting the models to assumptions of linearity (see below and Figure S3).

We also explored for biologically plausible combinations of clinically relevant predictive biomarkers. Examples included Ang1 or Ang2 with Tie2 and PlGF with VEGFR1 and VEGFR-2. Where combinations of biomarkers showed potential predictive characteristics, we tested for independence between combined versus individual biomarker models. Analyses were carried out using R software (version 2.7.1, The R development Core Team, R Foundation for Statistical Computing, Vienna, Austria) and STATA (version 11.1, College Station, Texas, USA) for the MFPI analyses.

**Multivariable Fractional Polynomials Interaction (MFPI) Models**

We investigated for interactions between treatment and continuous covariates using a multivariable fractional polynomials interaction, which is based on fractional polynomials (FP) methodology and provides a method of testing for continuous-by binary interactions and by modelling the treatment effect as a function of a continuous covariate. An FP function with one power term is known as an FP1 function. It takes the form *β*1*xp*1, with the power, *p*1, chosen from the set *S* = (*−*2*,−*1*,−*0*.*5*,* 0*,* 0*.*5*,* 1*,* 2*,* 3), where *x*0 denotes log *x*. An FP function with two power terms is called an FP2 function and takes the form *β*1*xp*1 + *β*2 *p*2 , with *p*1 and *p*2 both chosen from *S*. In the mathematical limit as *p*2 tends to *p*1, a so-called “repeated-powers” FP2 function is obtained, taking the form *β*1*xp*1 +*β*2*xp*2 log *x.* In all, there are 8 FP1 functions (including the linear function) and 36 FP2 functions (including eight repeated-powers functions). The best fitting model was determined from the Akaike’s information criterion (AIC).

We next visually checked for interactions among candidate biomarkers deriving treatment effect plots: first, a plot of the effect of biomarker on either treatment, together with a cumulative distribution function (CDF); and second, an estimated effect of the treatment (bevacizumab) according to the level of the biomarker. Experience has shown us that in interactive examples, where the different treatments and CDF coincide indicates an appropriate cut-off point.

We screened all 15 biomarkers for prediction associations reporting the interactive term from the MFPI cox regression modelling. There were no significant associations other than a borderline significance for Ang1 (chi2: 4.22, p = 0.040). With the exceptions of Tie2 and IL8, best models were those for linear representation of the biomarker variable.

Based on the screening and the biological link between Ang1 and Tie2 (Ang1 is the ligand for the Tie2 receptor), we explored these two candidates in more detail constructing treatment effect plots (see Figure 1 – main manuscript). For Ang1, there is a clear divergence of effect of the biomarker for standard versus bevacizumab treatments – the divergence approximates to the 50th percentile (p50). The treatment effect plot demonstrates that for Ang1 values greater than p50 are generally deleterious (p = 0.04). For Tie2, there is a no clear effect of the biomarker on standard versus bevacizumab treatments, but there is a general increase in hazard of adverse outcome with increasing Tie2 values. The treatment effect plot demonstrates that for Tie2, values greater than p50 are generally heterogeneous (we exploited this in the biomarker combination analyses).

**Table S6 – Tumour response by Ang1/Tie2 combination categories by treatment in the training set**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Standard arm (%)** |  | **Bevacizumab arm (%)** |  |
| Biomarker |  |  |  | P value\* |
|  |  |  |  |  |
| **High Ang1/low Tie2** |  |  |  |  |
| Non-responder | 4/9 (44) |  | 2/11 (18) |  |
| Responder | 5/9 (56) |  | 9/11 (82) | 0.202 |
| **High Ang1/high Tie2** |  |  |  |  |
| Non-responder | 7/16 (44) |  | 6/9 (67) |  |
| Responder | 9 /16 (75) |  | 3/9 (33) | 0.271 |
| **Low Ang1/any Tie2** |  |  |  |  |
| Non-responder | 8/19 (42) |  | 12/27 (44) |  |
| Responder | 11/19 (58) |  | 15/27 (56) | 0.875 |
|  |  |  |  |  |

Values in parentheses are percentages. \*Chi squared test

We explored tumour response in a logistic model to allow for inclusion of potential confounder variables. We took low Ang1/any Tie2 as the reference category. Similar patterns emerged. High Ang1/low Tie2 was associated with high odds of tumour response (odds ratio: 1.76) while high Ang1/high Tie2 is associated with a reduced odds of tumour response (odds ratio: 0.62) after adjustment for age, treatment, and performance status (ps).

Logistic regression

Number of obs = 90

LR chi2(6) = 3.39

Prob > chi2 = 0.7586

Log likelihood = -59.886012

Pseudo R2 = 0.0275

------------------------------------------------------------------------------

responder Odds Ratio Std. Err. Z P>|z [95% Conf. Interval]

-------------+----------------------------------------------------------------

bevacizumab 1.068 .4908 0.14 0.886 .4339 2.629

comb cat

1 1.762 1.026 0.97 0.331 .563 5.518

2 .6193 .3278 -0.91 0.365 .219 1.748

Age 1.021 .0255 0.82 0.413 .972 1.072

PS

1 .865 .397 -0.32 0.752 .352 2.125

2 .684 1.058 -0.25 0.806 .0329 14.211

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**Table S7 Cox proportional hazard models confirming Ang1 and Tie2 as a joint biomarker in the validation dataset (N = 114)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Covariates** | **Hazard Ratio** | **95% CI** | **p value** |
| **FIGO stage** |  |  | <0.105 |
| FIGO stage I | 1.000 | referent |  |
| FIGO stage II | 0.999 | 0.244-4.087 |  |
| FIGO stage III | 2.779 | 0.808-9.554 |  |
| FIGO stage IV | 2.905 | 0.724-11.658 |  |
| **Size of residual disease** |  |  |  |
| ≤1 cm residual disease | 1.000 | Referent |  |
| >1 cm residual disease | 4.359 | 2.469-7.696 | <0.001 |
| **Treatment** |  |  |  |
| Standard arm | 1.000 | Referent |  |
| Bevacizumab arm | 0.305 | 0.129-0.718 | 0.007 |
| **Individual biomarkers** |  |  |  |
| Ang1 |  |  |  |
| < median | 1.000 | Referent |  |
| ≥ median | 0.662 | 0.702-4.073 | 0.402 |
| Tie2 |  |  |  |
| ≥ median | 1.000 | referent |  |
| < median | 1.427 | 0.253 – 1.737 | 0.377 |
| **Interaction terms\*** |  |  |  |
| Ang1 \* Treatment | 1.418 | 0.455 -4.424 | 0.547 |
| Tie2 \* Treatment | 3.199 | 0.928-11.030 | 0.066 |
| Ang1 \* Tie2 | 1.183 | 0.229-6.109 | 0.841 |
| Ang1 \* Tie2 \* Treatment | 0.179 | 0.021-1.524 | 0.115 |
|  |  |  |  |

There were no missing data.

\*In all models, standard arm, 0; bevacizumab arm, 1; ang1 < median, 0; ang1 ≥ median, 1; tie2 < median, 1; tie2 ≥ median, 0. All interaction terms are multiplicative.