Supplementary Materials

Supplementary Figure 1. Outcome of the Animal Tumor Assays from the Results of the Morphologic Assays

A model for predicting the outcomes of animal tumor assays from the results of morphologic assays was constructed. A separate analysis was performed for each of the three classes of animal tumor assays (general, colon, mammary) using results of the six morphologic assays as predictors. Both animal tumor and morphologic assays generated ordinal data (values of 0, 1, 2, 3) (**Table 2**), so ordinal logistic regression was applied. Under this model, if Y is the potential outcome of the animal tumor assay and X_{ACF}, X_{A427}, X_{HFE}, X_{JB6}, X_{MMOC}, and X_{RTE} are the observed outcomes of the morphologic assays, the logarithm of the odds that Y will be at most j, where j ranges from 0 to 2, is $a_j + b_{ACF} X_{ACF} + b_{A427} X_{A427} + b_{HFE} X_{HFE} + b_{JB6} X_{JB6} + b_{MMOC} X_{MMOC} + b_{RTE} X_{RTE}$. Note that the slope coefficients (the b's) do not change with j; only the intercept a changes.

We allowed for options in which certain morphologic assays had not been used and were therefore not available for incorporation in the model. Consideration of all possible such options yielded a set of 63 models (**Supplementary Figure 1**). Furthermore, since a relatively small number of outcomes of animal tumor assays had associated results from all six morphologic assays, multiple imputation was performed to generate multiple complete data sets having close concordance with the observed data. The number of imputed data sets was 100. For every imputed data set, each of the 63 ordinal logistic regression models was fit. Then, for every model, the SAS procedure MIANALYZE was applied to obtain combined estimates of the parameters (the a's and b's) over the 100 data sets with associated standard errors. This yielded 63 models with their estimated parameters. These

63 models were compared using a bias-corrected version (AICc) of the Akaike Information Criterion (AIC), which discourages overfitting. The AICc values were used to obtain parameter estimates that were AICc-weighted averages of the estimates over all 63 models, a technique known as multimodel inference.

Using multimodel inference two models – the general model (**Supplementary Figure 1A**) and the mammary-specific model (**Supplementary Figure 1B**) were generated. The equations depicting the models are presented in terms of a distribution for Y instead of the logarithms of odds. A comparable model for predicting colon-specific outcomes from the results of the morphologic assays is presented in **Figure 3**.

Supplementary Figure 1A. General Model for Predicting the Outcome of the Animal Tumor Assays from the Results of the Morphologic Assays

Supplementary Figure 1B. Mammary-specific Model for Predicting the Outcome of the Animal Tumor Assays from the Results of the Morphologic Assays

Figure 1a

$$P(Y=0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(0.849-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)}{1+\exp\left(0.849-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)}$$

$$P(Y=1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(1.849-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)}{1+\exp\left(1.849-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)} \;-$$

$$P(Y=0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE})$$

$$P(Y=2 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(2.538-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)}{1+\exp\left(2.538-0.559x_{ACF}+0.019x_{A\,427}-0.159x_{HFE}-0.031x_{JB\,6}-0.288x_{MMOC}-0.153x_{RTE}\right)} \;-$$

$$P(Y=0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) - P(Y=1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE})$$

$$P(Y=3 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) \quad =$$

$$1 - P(Y=0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) -$$

$$P(Y=1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE}) - P(Y=2 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB\,6}, x_{MMOC}, x_{RTE})$$

Figure 1b

$$P(Y = 0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(1.521 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)}{1 + \exp\left(1.521 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)}$$

$$P(Y = 1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(2.891 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)}{1 + \exp\left(2.891 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)} \quad -$$

$$P(Y = 0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})$$

$$P(Y = 2 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) \quad =$$

$$\frac{\exp\left(4.065 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)}{1 + \exp\left(4.065 - 0.031 x_{ACF} - 0.205 x_{A\,427} - 0.329 x_{HFE} - 0.010 x_{JB6} - 0.428 x_{MMOC} - 0.111 x_{RTE}\right)} \quad -$$

$$P(Y = 0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) - P(Y = 1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})$$

$$P(Y = 3 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) \quad =$$

$$1 - P(Y = 0 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) -$$

$$P(Y = 1 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE}) - P(Y = 2 \mid x_{ACF}, x_{A\,427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})$$

Supplementary Figure 2. Equations predicting the probability of a positive tumor result (y-axis) in each of the three animal tumor models based on individual morphologic assay rank results, while holding the outcomes for the other five assays at 0. These equations were used to generate the curves in **Figure 4**.

Figure 2

General Model

$$P(Y>0|x_{ACF}, x_{A427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})=$$

$$\frac{1}{1+\exp(0.849-0.559x_{ACF}+0.019x_{A427}-0.159x_{HFE}-0.031x_{JB6}-0.288x_{MMOC}-0.153x_{RTE})}$$

Colon Model

$$P(Y>0|x_{ACF}, x_{A427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})=$$

$$\frac{1}{1+\exp(0.410-0.698x_{ACF}-0.092x_{A427}-0.015x_{HFE}-0.076x_{JB6}+0.002x_{MMOC}-0.075x_{RTE})}$$

Mammary Model

$$P(Y>0|x_{ACF}, x_{A427}, x_{HFE}, x_{JB6}, x_{MMOC}, x_{RTE})=$$

$$\frac{1}{1+\exp(1.521-0.031x_{ACF}-0.205x_{A427}-0.329x_{HFE}-0.010x_{JB6}-0.428x_{MMOC}-0.111x_{RTE})}$$

Supplementary Table 1. Numbers and Percentage of Agents Grouped by Each Morphologic - Animal Tumor Assay Pair (Table 1A) and Grouped by Combination of Morphologic Assays - Animal Tumor Assay Pairs (Table 1B). Columns and rows labeled "Yes" indicate the number (N) and percentage (%) of agents that have been tested in the specified combination of assays. Columns and rows labeled "No" point out the N and % of agents not tested in the indicated combination. "Any Tumor Assay" refers to animal tumor assays reflecting cancers at all sites, not only colon and mammary. Similarly, "Any Morphologic Assay" reflects the number of agents tested in at least one of the morphologic assays. **Supplementary Table 1A** shows the number of agents tested (or not) in pairwise combinations of morphologic and tumor assays. For example, of the 210 agents in any morphologic and any tumor assay, 91 have and 119 have not been tested in a colon tumor assay. Of the 91 tested in a colon tumor assay, 90 have been tested in the ACF assay, 68 in A427, and 42 in HFE. **Supplementary Table 1B** shows the subset of combinations of morphologic assays and tumor assays that exist in the data set. Of the 63 possible combinations, 30 are represented in the data. For example, where the number of morphologic assays equals 1, 4 of the 6 possible scenarios are shown. The two remaining individual assays were not done in the absence of other morphologic assays; no agents were tested in either HFE or JB6 and not tested in a second morphologic assay. These two combinations are therefore omitted from the table. In total, 30 possible combinations were carried out and 33 were not and are omitted from **Table 1B**.

Supplementary Table 1A. Number and Percentage of Agents Tested, Grouped by Individual Morphologic Assay and Animal Tumor Assay ("Yes" refers to agents tested and "No" refers to agents not tested.)

		Colon tumor assay				Mammary tumor assay				Any tumor assay	
		Yes		No		Yes		No		Yes	
		N	%	N	%	N	%	N	%	N	%
Any morphologic assay		91	100	119	100	146	100	64	100	210	100
ACF	Yes	90	98.9	65	54.6	112	76.7	43	67.2	155	73.8
	No	1	1.1	54	45.4	34	23.3	21	32.8	55	26.2
A427	Yes	68	74.7	80	67.2	112	76.7	36	56.3	148	70.5
	No	23	25.3	39	32.8	34	23.3	28	43.8	62	29.5
HFE	Yes	42	46.2	17	14.3	48	32.9	11	17.2	59	28.1
	No	49	53.8	102	85.7	98	67.1	53	82.8	151	71.9
JB6	Yes	35	38.5	39	32.8	68	46.6	6	9.4	74	35.2
	No	56	61.5	80	67.2	78	53.4	58	90.6	136	64.8
MMOC	Yes	67	73.6	85	71.4	119	81.5	33	51.6	152	72.4
	No	24	26.4	34	28.6	27	18.5	31	48.4	58	27.6
RTE	Yes	75	82.4	92	77.3	124	84.9	43	67.2	167	79.5
	No	16	17.6	27	22.7	22	15.1	21	32.8	43	20.5

Supplementary Table 1B. Number and Percentage of Agents Tested, Grouped by Combination of Morphologic Assays and Animal Tumor Assay ("Yes" refers to agents tested and "No" refers to agents not tested.)

# Morph	ACF	A 427	HFE	JB6	MMOC	RTE	Colon tumor assay Yes N	%	No N	%	Mammary tumor assay Yes N	%	No N	%	Any tumor assay Yes N	%
1	No	No	No	No	No	Yes	—	—	9	7.6	3	2.1	6	9.4	9	4.3
1	No	No	No	No	Yes	No	1	1.1	9	7.6	7	4.8	3	4.7	10	4.8
1	No	Yes	No	No	No	No	—	—	1	0.8	—	—	1	1.6	1	0.5
1	Yes	No	No	No	No	No	11	12.1	8	6.7	8	5.5	11	17.2	19	9
2	No	No	No	No	Yes	Yes	—	—	1	0.8	—	—	1	1.6	1	0.5
2	No	No	No	Yes	No	Yes	—	—	2	1.7	1	0.7	1	1.6	2	1
2	No	No	Yes	No	No	Yes	—	—	1	0.8	1	0.7	—	—	1	0.5
2	No	Yes	No	No	No	Yes	—	—	2	1.7	1	0.7	1	1.6	2	1
2	No	Yes	No	No	Yes	No	—	—	3	2.5	1	0.7	2	3.1	3	1.4
2	Yes	No	No	No	No	Yes	6	6.6	5	4.2	8	5.5	3	4.7	11	5.2
2	Yes	No	No	No	Yes	No	—	—	1	0.8	1	0.7	—	—	1	0.5
2	Yes	Yes	No	No	No	No	—	—	1	0.8	1	0.7	—	—	1	0.5
3	No	No	No	Yes	Yes	Yes	—	—	1	0.8	1	0.7	—	—	1	0.5
3	No	Yes	No	No	Yes	Yes	—	—	13	10.9	8	5.5	5	7.8	13	6.2
3	No	Yes	No	Yes	Yes	No	—	—	1	0.8	—	—	1	1.6	1	0.5
3	No	Yes	Yes	No	No	Yes	—	—	1	0.8	1	0.7	—	—	1	0.5
3	Yes	No	No	Yes	Yes	Yes	2	2.2	1	0.8	2	1.4	1	1.6	3	1.4
3	Yes	No	Yes	No	No	Yes	1	1.1	1	0.8	—	—	2	3.1	2	1
3	Yes	Yes	No	No	No	Yes	4	4.4	3	2.5	3	2.1	4	6.3	7	3.3
3	Yes	Yes	No	No	Yes	No	3	3.3	1	0.8	2	1.4	2	3.1	4	1.9
4	No	Yes	No	Yes	Yes	Yes	—	—	9	7.6	9	6.2	—	—	9	4.3
4	Yes	No	No	Yes	Yes	Yes	2	2.2	—	—	2	1.4	—	—	2	1
4	Yes	Yes	No	No	Yes	Yes	10	11	15	12.6	18	12.3	7	10.9	25	11.9
4	Yes	Yes	No	Yes	Yes	No	—	—	2	1.7	1	0.7	1	1.6	2	1
4	Yes	Yes	Yes	No	No	Yes	2	2.2	—	—	—	—	2	3.1	2	1
4	Yes	Yes	Yes	Yes	No	No	1	1.1	—	—	1	0.7	—	—	1	0.5
5	No	Yes	Yes	Yes	Yes	Yes	—	—	1	0.8	1	0.7	—	—	1	0.5
5	Yes	Yes	No	Yes	Yes	Yes	10	11	14	11.8	21	14.4	3	4.7	24	11.4
5	Yes	Yes	Yes	No	Yes	Yes	15	16.5	4	3.4	12	8.2	7	10.9	19	9
6	Yes	Yes	Yes	Yes	Yes	Yes	23	25.3	9	7.6	32	21.9	—	—	32	15.2