Considerations in the Development and Validation of Genomic Tests for Cancer Recurrence and Treatment Benefit

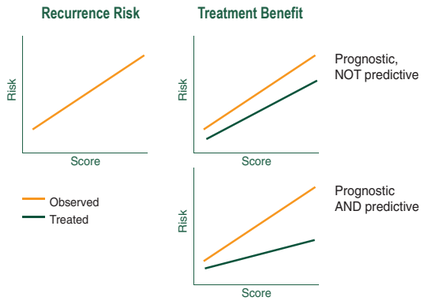
D.J. Kerr[1], M.J. O'Connell[2], I.C. Lavery[3], R.G. Gray[4], G. Yothers[2], P. Quirke[5], M. Lopatin[6], K.M. Clark-Langone[6], M. Lee[6], and N. Wolmark[2]

1. University of Oxford, Oxford, UK; 2. National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, PA, USA; 3. Cleveland Clinic, Cleveland, OH, USA;
4. Birmingham Clinical Trials Unit, Birmingham, UK; 5. Leeds Institute of Molecular Medicine, Leeds, UK; 6. Genomic Health, Inc., Redwood City, CA, USA

Overview and Objectives

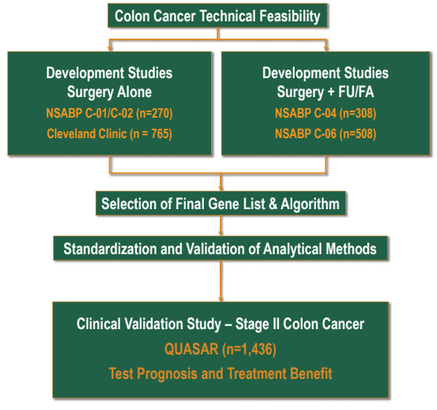
- The development of standardized, clinically validated genomic tests to determine recurrence risk and treatment benefit for individual cancer patients is challenging
- To illustrate specific considerations encountered in the development and validation of assays for recurrence risk and treatment benefit, we compare results from the development and validation studies for
  - The 12-gene colon cancer Recurrence Score (RS), designed to quantitate individual recurrence risk in stage II colon cancer patients following surgery, and
  - The 11-gene Treatment Score (TS), designed to identify individual colon cancer patients with differential relative risk reduction with adjuvant fluorouracil/folinic acid (FU/FA)

Figure 1. Relationship of Marker to Recurrence Risk and Treatment Benefit



- To evaluate relationship of marker to treatment benefit
  - Requires samples from a randomized study[4]
  - Compared to recurrence risk, requires typically at least 4 times as many patients to achieve same statistical power for test of interaction

Figure 2. Multi Step Development and Validation Strategy for a Multi-Gene RT-PCR Colon Cancer Assay[2]



Methods

- This study presents a comparison of results of analyses of the Development (DEV) and Validation (VAL) studies performed according to prospectively-designed study protocols and statistical analysis plans

Laboratory Methods

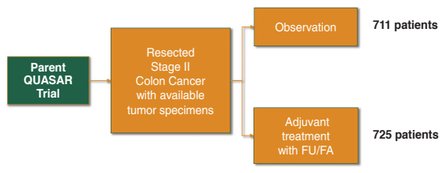
- For DEV studies, quantitative RT-PCR for 761 (or 375) candidate genes (including the genes corresponding to the 12-gene Recurrence Score and the 11-gene Treatment Score) was performed from fixed, paraffin-embedded colon tumor tissue from stage II and stage III colon cancer patients, as previously described.[1]
- For the VAL study, quantitative RT-PCR for the 12-gene Recurrence Score and the 11-gene Treatment Score (including the 5 reference genes in common) was performed from fixed, paraffin-embedded colon tumor tissue from stage II colon cancer patients from the QUASAR clinical trial, as previously described.[2]

Table 1. Colon Cancer Assay Development Studies: Gene Discovery and Gene Refinement[1]

Treatment	Study	# Patients (Stage II/III)	# Genes
Surgery Alone	C01/C02, NSABP, Pittsburgh, PA	270 (131/139)	761
Surgery Alone	Cleveland Clinic, Cleveland, OH	765 (504/261)	375
Surgery plus FU/FA	C04, NSABP, Pittsburgh, PA	308 (137/171)	761
Surgery plus FU/FA	C06 NSABP, Pittsburgh, PA	508 (235/273)	375

- Association between gene expression and recurrence-free interval (RFI) across four independent studies. Total of 1851 patients.
- All single arm studies, and thus gene expression by treatment interaction had to be inferred by comparisons across studies

Figure 3. Prospectively-Designed Clinical Validation Study: Stage II Colon Cancer Patients from QUASAR[2]



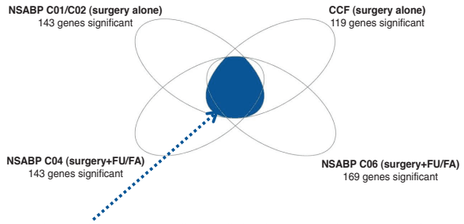
Quantitative RT-PCR for pre-specified 12-gene Recurrence Score and 11-gene Treatment Score

Statistical Methods

- Cox regression models were used to assess the association of gene expression with recurrence-free interval (RFI), the primary clinical endpoint, and differential treatment benefit with adjuvant FU/FA.
  - Analyses of disease-free survival and overall survival were included as secondary endpoints.
- While data for both stage II and stage III patients were used in the development of the RS and TS, the performance of the scores is reported here for stage II patients only, for consistency with the QUASAR validation study

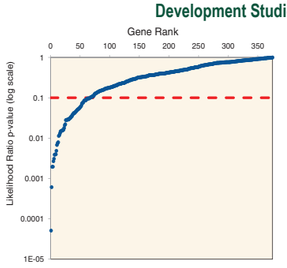
Results

Figure 4. Identification of Recurrence Genes in Development Studies



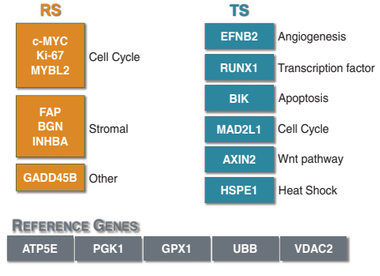
- 48 (13%) of 375 genes studied in all development studies were significantly (p<0.05) associated with RFI in both surgery alone and at least one surgery + FU/FA study
  - <1 gene expected to be a false discovery

Figure 5. Identification of FU/FA Benefit Genes in Development Studies



- 66 (18%) of 375 genes studied in all development studies had significant (p<0.1) interaction between gene expression and FU/FA treatment when data across four studies were pooled
  - 37 genes expected to be false discoveries

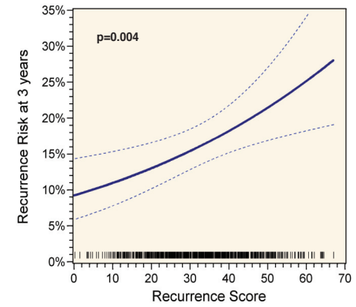
Figure 6. Final Pre-Specified 12-Gene Recurrence Score and 11-Gene Treatment Score



- The final 7 recurrence genes and 6 FU/FA benefit genes were selected using a number of considerations including:
  - strength of associations with recurrence
  - involvement in biological pathways functionally important in colon cancer
  - consistency of performance across studies
  - consistency of performance in stage II and stage III patients
  - analytical performance
  - dynamic range of expression

Figure 7. QUASAR Results: 12-Gene Recurrence Score Predicts Recurrence Following Surgery[2]

Prospectively-Defined Analyses in Stage II Colon Cancer (n=711)



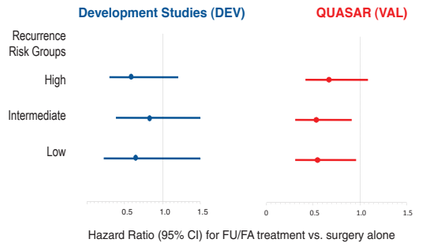
RS is significantly associated with RFI[2]

Study	Two-sided p-value
DEV	<0.001
VAL	**0.004**

RS is significantly associated with RFI after controlling for clinical and pathological covariates[2]

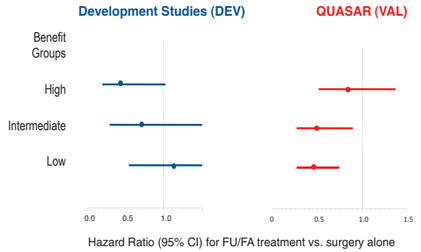
Study	two-sided p-value
DEV	<0.001
VAL	**0.008**

Figure 8. Prediction of Differential FU/FA Benefit: Recurrence Score



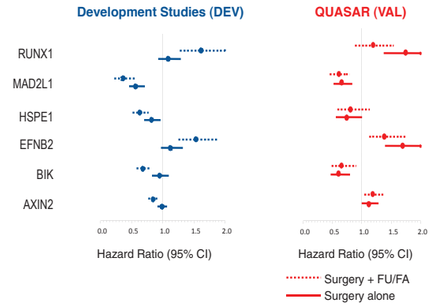
- RS by Treatment interaction for RFI: p=0.39 DEV vs. p0.76 VAL
- Similar proportional risk reduction observed with FU/FA across risk groups in QUASAR validation study (one-sided p=0.69)

Figure 9. Prediction of Differential FU/FA Benefit: Treatment Score



- TS by Treatment interaction for RFI: p=0.010 DEV vs. p0.19 VAL
- No trend in absolute benefit in pre-specified direction across benefit groups in the QUASAR validation study (one-sided p=0.95)

Figure 10. Prediction of Differential FU/FA Benefit: Individual Genes



- 1 TS gene showed a significant interaction w/ treatment in QUASAR (RUNX1, p<0.05) but in direction opposite to that in the DEV studies
- Most TS genes had similar relationship with recurrence risk in both patients treated with surgery alone and surgery + FU/FA

Strengths & Limitations

Strengths

- Multiple large, independent studies for discovery of recurrence and predictive genes
- Prospectively-designed validation study in large, randomized clinical trial
- Use of standardized, analytically validated assay platform
- Highly effective for defining genomic determinants of recurrence risk

Limitations

- Use of single arm studies for development
  - Differences between study populations (i.e. in separate surgery alone vs. surgery + FU/FA studies) may have confounded discovery of predictive genes
- Limited statistical power in development studies for identification of predictive genes and in validation study for test of interaction
- Molecular markers of FU/FA treatment benefit potentially not included in discovery effort

Summary and Conclusions

- This strategy, involving more than 3000 patient specimens, successfully validated genes for colon cancer recurrence but not FU/FA benefit.
  - The Recurrence Score was validated as an independent predictor of recurrence risk for individual stage II colon cancer patients. Patients with high Recurrence Score disease should be considered more strongly for adjuvant therapy due to higher recurrence risk and thus larger absolute risk reduction with FU/FA.
- Investigation of predictive genes in this study may have been limited in part by lower statistical power for identification of these genes as well as use of non-randomized comparisons in DEV studies.

References

1. O'Connell MJ, Lavery I, Yothers G, et al. Relationship between tumor gene expression and recurrence in four independent studies of stage II/III colon cancer patients treated with surgery alone or surgery plus adjuvant 5-FU/LV. J Clin Oncol 2010; 28:3937-3944.
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3. Clark-Langone KM, Wu JY, Sangli C, et al: Biomarker discovery for colon cancer using a 761 gene RT-PCR assay. BMC Genomics 2007; 8:279-297.
4. Mandrekar, , S.J. and Sargent, D.J. Clinical trial designs for predictive biomarker validation: Theoretical considerations and practical challenges. J Clin Oncol 2009; 27:4027-4034.

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