Background and Methods

A series of studies are needed to identify genes that are predictive of clinical outcomes and then to validate that they are reliable and clinically useful.

Development Process for a Genomic Assay

This assay was successfully used to develop the Onco

Figure 2. Candidate genes.

• Candidate genes: 757 genes (from literature, biologic pathways, microarray experiments) (Figure 3)
• Median follow-up 15.7 years

Figure 1. This study was designed to identify genes whose expression levels are associated with clinical outcomes.

Results

Analysis of Gene Co-expression and Biologic Function

Figure 5. Relationship Between Tumor Gene Expression and Recurrence in Stage II/III Colon Cancer: Literature

Table 1. Evaluation of Clinical Relevance

Relationship Between Tumor Gene Expression and Recurrence in Stage II/III Colon Cancer: Quantitative RT-PCR Analysis of 757 Genes in Fixed Paraffin-Embedded (FPE) Tissue

Multivariate Analysis

Table 2. Preliminary Identification of Tumor Gene Expression and Their Relationship to Recurrence in Stage II/III Colon Cancer

CONCLUSIONS

Acknowledgements

REFERENCES

Table 3. Estimation of Clinical Relevance

Table 4. Preliminary Results

Figure 4. Performance of RT-PCR Assay After Multiple Iterations of Assay Development
Relationship Between Tumor Gene Expression and Recurrence in Stage II/III Colon Cancer: Quantitative RT-PCR Assay of 757 Genes in Fixed Paraffin-Embedded (FPE) Tissue

Methodology

- **NSABP C-01/C-02 Study**
  - **Endpoints**
    - Primary: RFI
    - Secondary: DRFI, DFS, OS

- **Patient Selection**
  - **Patients:** surgery only or surgery + BCG arms of NSABP C-01/C-02

- **Development Process for a Genomic Assay**
  - The RT-PCR assay works on FPE tumor tissue and therefore can be used on tissues archived from past studies.
  - Genomic Health has developed a real-time PCR (RT-PCR) assay that quantifies gene expression (RNA levels) in fixed paraffin-embedded (FPE) tumor tissue.1

- **Candidate Gene Selection**
  - **Candidate Genes:** 757 genes (from literature, biologic pathways, microarray experiments) (Figure 3)

- **Univariate Analysis of Clinical Variables and Outcome**
  - **LAMA3 Expression (Log2)**

- **Table 1.** Hazard Ratio in C-04

**END OF TEXT**
This RT-PCR technology has now been successfully applied in colon cancer.

**Candidate genes**: 757 genes (from literature, biologic pathways, microarray experiments) (Figure 3)

**Blocks**: 270 evaluable patients

This study was designed to identify genes whose expression levels are associated with clinical outcomes.

Similar to drug development, there is a stepwise approach to development of a clinical diagnostic assay (Figure 2).

**This assay was successfully used to develop the Onco**

- **Secondary**: distant RFI (DRFI), disease-free survival (DFS), overall survival (OS)
- **Primary**: recurrence-free interval (RFI)

**STUDY DESIGN**

**OBJECTIVE**

Development Process for a Genomic Assay Including Example Studies from the Colon Cancer Program

**Gene Identification and Refinement Studies**

- Surgery Alone: NSABP Studies C-01/C-02
- Prognosis and Treatment Benefit

**Technical Feasibility**

- Clinical Validation

**Univariate Analysis of Gene Expression and Outcome**

- Gene expression was successfully measured for 753 of the 757 genes.

**Univariate Analysis of Clinical Variables and Outcome**

- Comparison, stratification by stage results in a 5-year risk of recurrence of 29% for stage II patients and 50% for stage III patients.

**Discriminate patients with different risks of recurrence (Figure 5). Stage II and III patients with lower levels of expression (1st tertile) had a 24% risk of recurrence**

**REFERENCES**


**Conclusion**

- Expression analysis of RT-PCR gene expression can reliably predict recurrence in colon cancer.
- Expression analysis of RT-PCR gene expression can be used to predict presence of distant recurrence.