

HLA TYPING AND EXPRESSION: POTENTIAL MARKER FOR IDENTIFYING EARLY DYSPLASIA AND STRATIFYING THE RISK FOR IBD-CANCER

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BACKGROUND

Patients suffering from ulcerative colitis (UC), one of two forms of inflammatory bowel disease (IBD), have an increased risk for developing colorectal cancer (CRC). The risk of CRC in the setting of UC is approximately twice that of the general population, and CRC remains the major cause of mortality by malignant disease in UC patients. Because of this considerable risk, regular endoscopic surveillance is recommended every 1-2 years to detect early precancerous (dysplastic) changes.¹

Surveillance programs have limitations. First, CUC/CRC often begins in flat, macroscopically indistinguishable mucosal lesions which therefore may not be identified and chosen for biopsy. Second, even with aggressive biopsy protocols, only a small portion of the colon is actually selected for pathological review, increasing the risk for “sampling” error. Finally, the diagnosis of low grade dysplasia, especially in the setting of active inflammation, can be difficult. As a result, patients often have advanced disease at diagnosis and overall survival rates remain low.^{1,2} These issues highlight the need to identify additional markers that can be used to stratify patients at greatest risk for developing CRC in the setting of longstanding UC.

Linkage analysis and twin studies have implicated a genetic component of CUC and have consistently shown linkage to chromosome 6.³ Given both their location on chromosome 6 and their role in the immune/inflammatory response, genes of the major histocompatibility complex, HLA Class I and Class II, and tumor necrosis factor alpha (TNF- α) are excellent candidate genes for identifying patients at increased risk for developing CUC/CRC. Previous studies of both HLA and TNF- α have shown associations between specific HLA types/TNF- α polymorphisms and risk for or protection from CUC, extent of disease, and extraintestinal manifestations.^{4,5} However, there has been limited investigation into the role that these genes may play in the risk for developing CRC.

GOALS & HYPOTHESES

1. To perform a preliminary investigation into HLA allele frequencies in patients with longstanding (10+ years) CUC complicated by CRC. We hypothesize that there will be a specific HLA type closely associated with CUC/CRC that is not seen among patients with CUC who do not develop CRC.
2. To analyze polymorphisms of TNF- α in patients with longstanding CUC who have not developed cancer and CUC/CRC patients. We hypothesize that certain polymorphisms will be associated with both CUC/CRC and with increased TNF- α protein expression *in situ*.
3. To investigate alterations of HLA Class I/II expression and T-cell populations within the affected colon of patients with CUC/CRC to determine if any changes observed within the tumor reflect changes that are occurring in the colon overall (macro versus microenvironment). We hypothesize that normal resection margins from patients with CUC/CRC will have expression patterns significantly different from patient samples of individuals with CUC/no dysplasia.

MATERIALS & METHODS

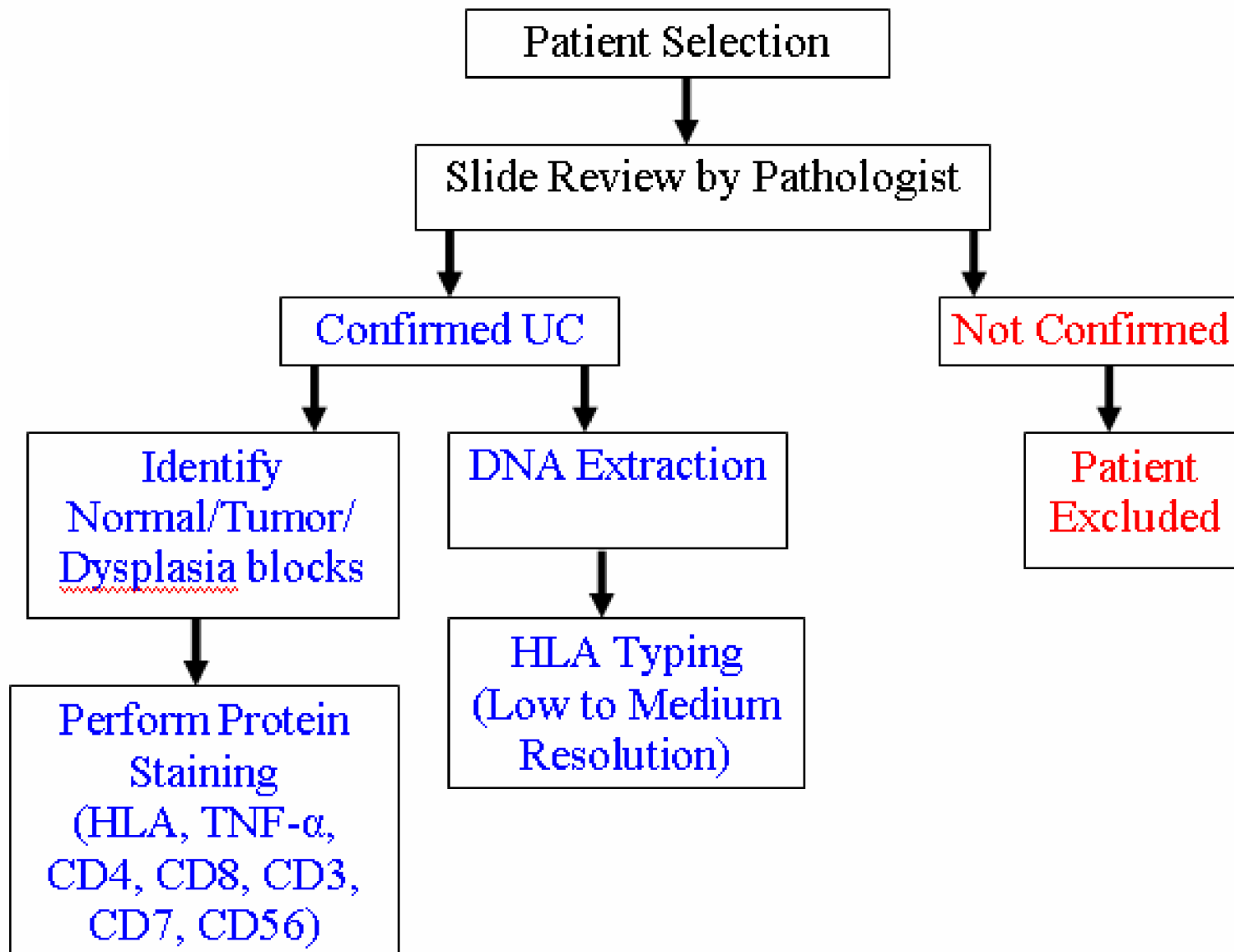
Chart Review:

To avoid the issues associated with previous studies of HLA type in IBD, we are performing an extensive chart review to obtain a strong sample set for these initial studies.⁶ In addition, we will also seek to identify other possible risk factors from both the patient and family history contained within each chart. The chart review will include:

1. Confirmation of initial diagnosis to exclude patients with < 10 years of CUC and avoid including sporadic CRC occurring in the setting of UC,
2. Efficacy of surveillance (i.e. how many have had biopsies on a regular basis),
3. Treatments and response,
4. Family (grandparents, parents, siblings and offspring) medical history,
5. Patient social and medical history.

This will be compiled into a comprehensive database that will be the core for this project as well as other studies of UC.

Testing Strategy

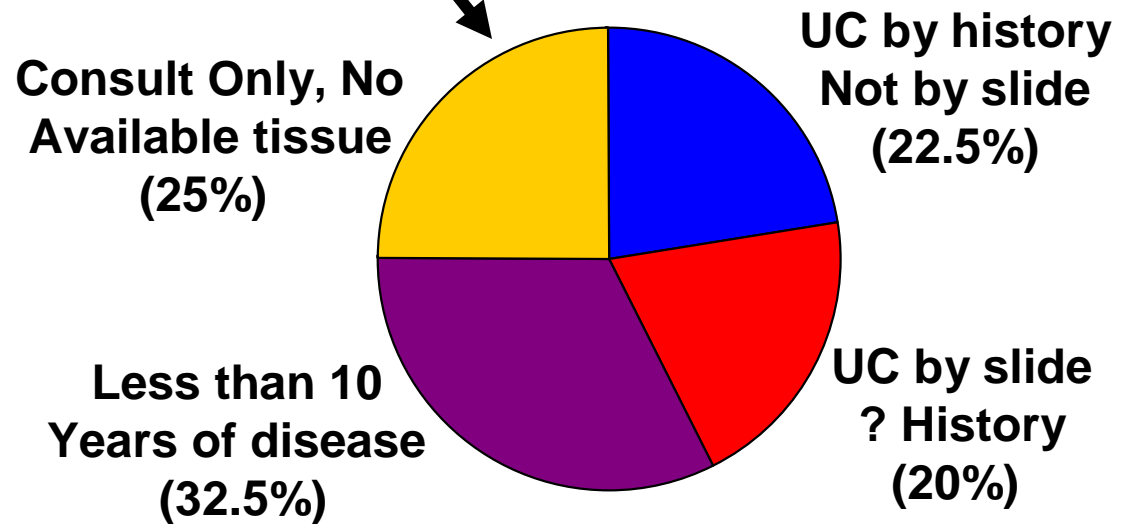
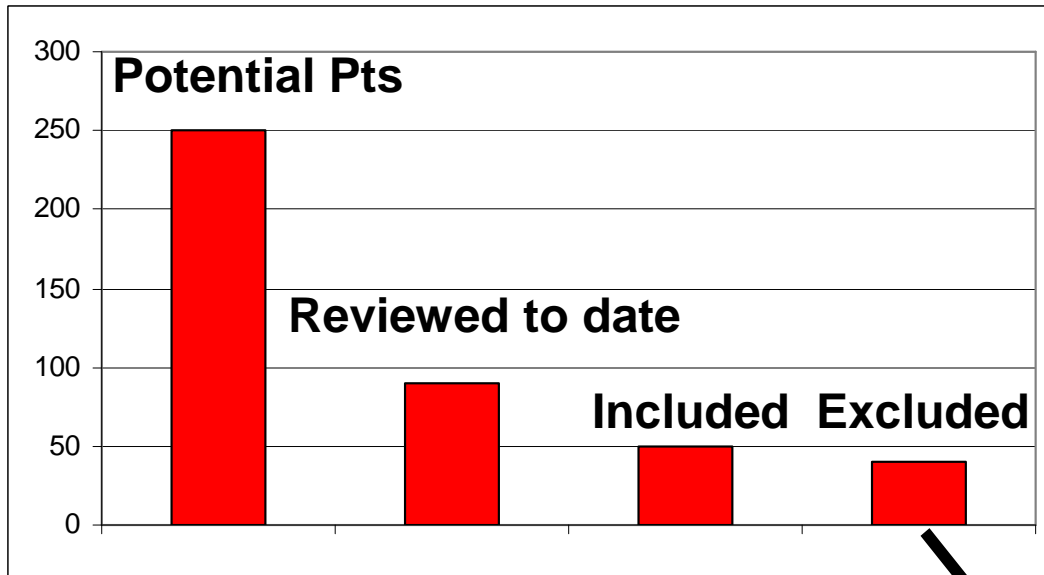


RESULTS

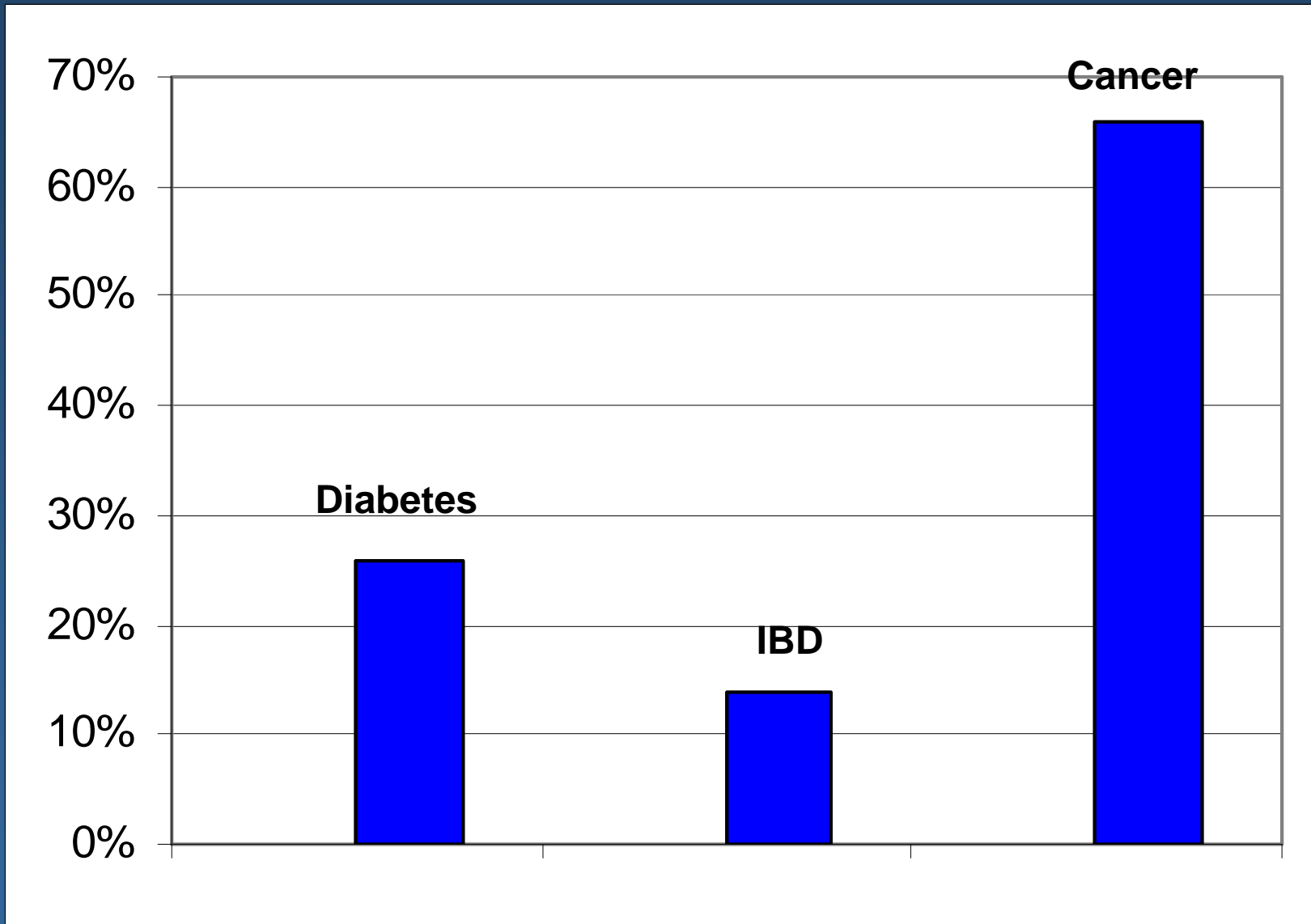
Chart Review:

The average age at the time of CRC diagnosis for the included patients (n=50) is 50.1 ± 11.6 (range 26-74) with an average disease duration of 20.7 ± 9.1 (range 10-49) years. There have been 11 reported deaths, 9 of which occurred in ≤ 2 years from CRC diagnosis. Stage of disease (Stage 1 or 2 vs 3 or 4) was similar (42% vs 58%). Ten patients had a previous biopsy (≤ 2 years prior to the CRC diagnosis) that did not indicate any dysplasia.

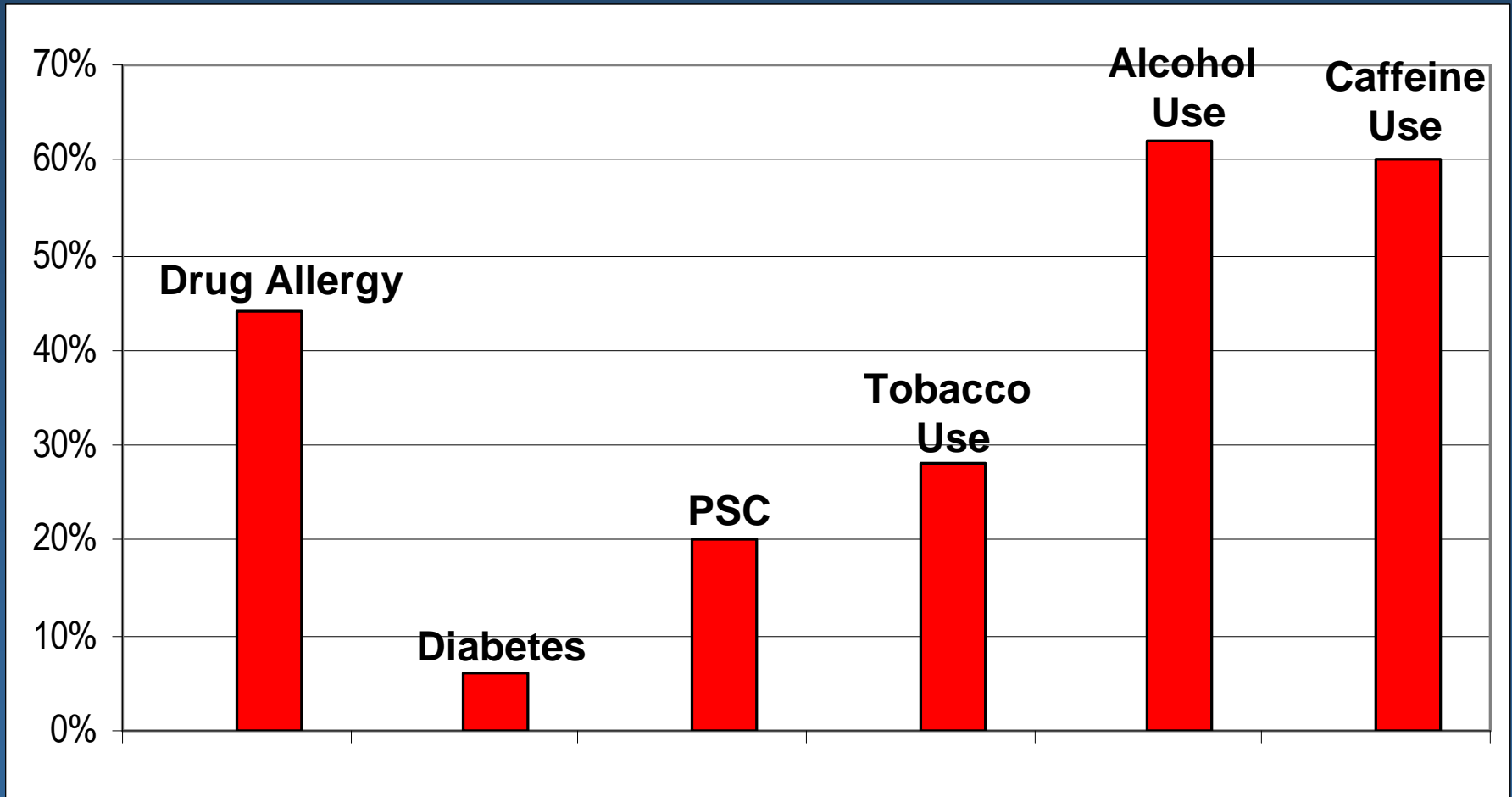
Chart Review Summary



Reported Family History



Reported Patient History



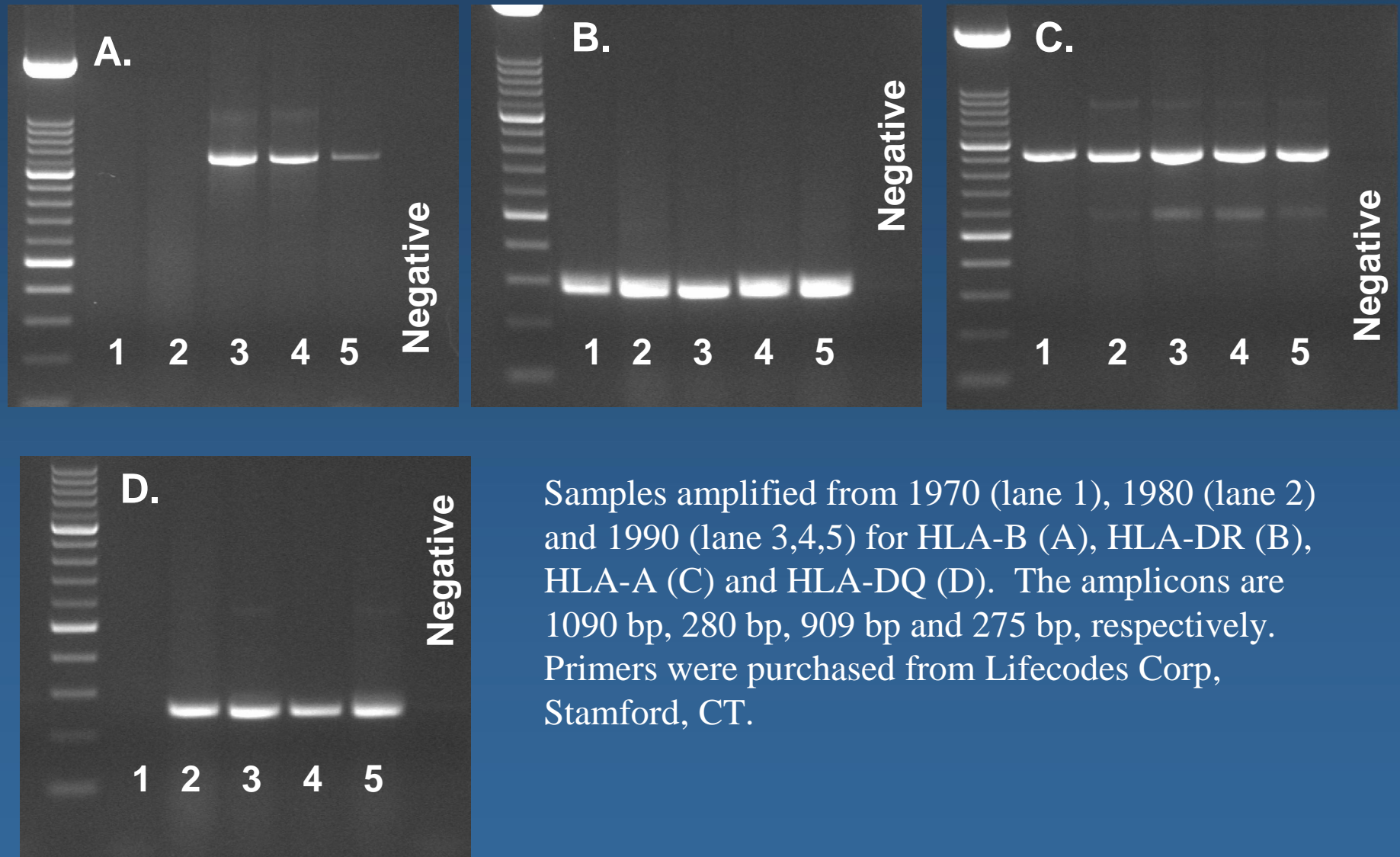
DNA Extraction and HLA Typing:

Because of the retrospective nature of this study, the DNA will come from formalin fixed, paraffin embedded tissues only. This is a very challenging material to deal with and reports of successful PCR amplification of DNA from samples over 10 years old are rare. The amplicon required to do HLA typing is large (>1000bp), adding to the difficulty.

The majority of our cases will be from the 1990's (< 10 years); however a subset will be from the 1980's and 1970's. We therefore have developed an extraction protocol that will provide adequate DNA to perform all the proposed testing and that will allow for amplification of the HLA loci. We used a test set of patients (6 from the 1970's, -80's and -90's) to optimize the extraction and then attempted HLA loci amplification.

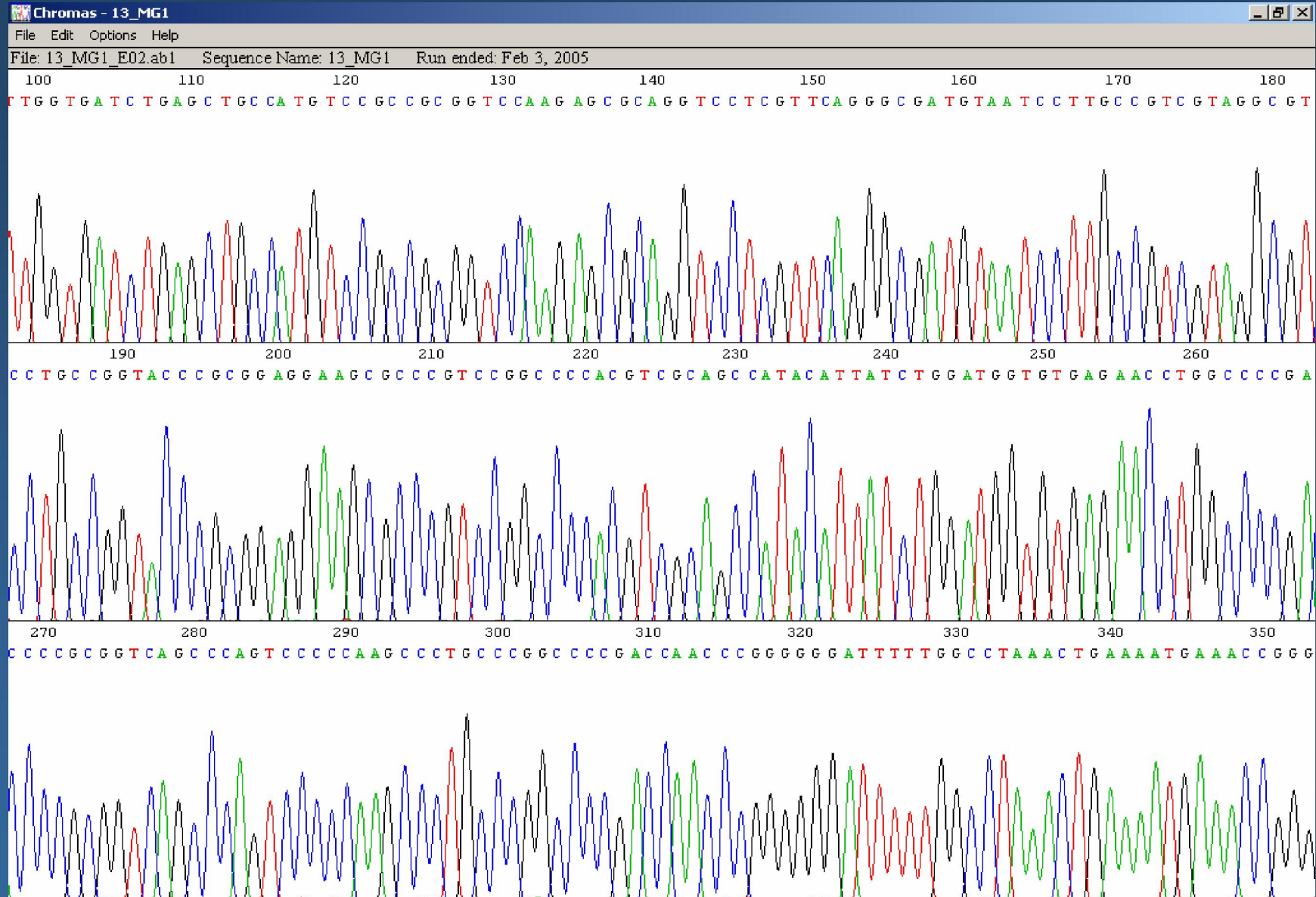
We successfully amplified virtually all loci for the 1990 samples (only one B locus failed), >50% of the loci for the 1980 samples (of note, 2 samples were extracted from lymph node tissue and yielded very low DNA concentrations), and ≈30% of the loci for the 1970 samples. Taken together, we are in an excellent position to utilize most of the patients that will be identified in this study for both HLA typing and TNF- α polymorphism analysis.

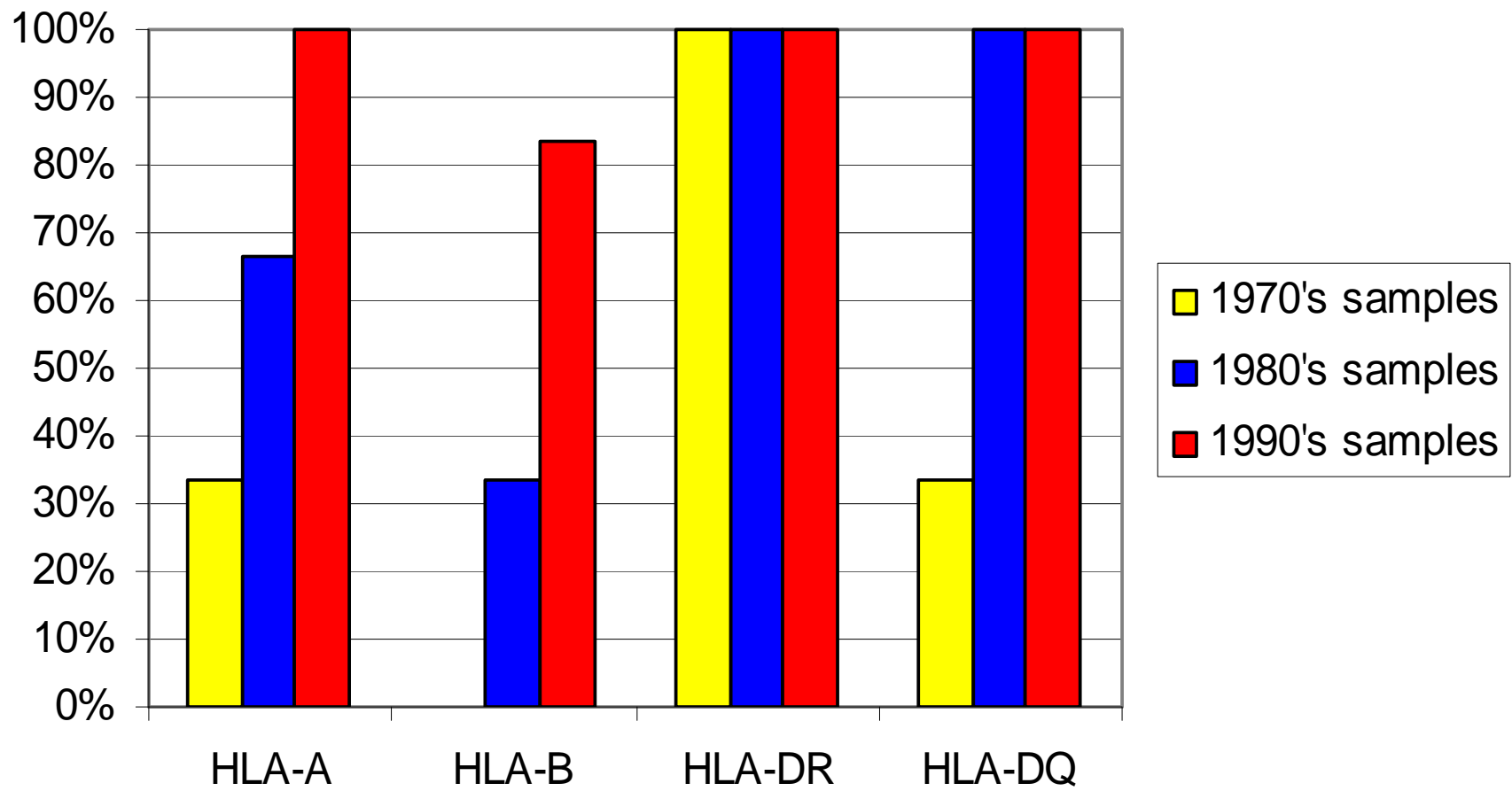
Demonstrated Amplification of HLA Loci



Samples amplified from 1970 (lane 1), 1980 (lane 2) and 1990 (lane 3,4,5) for HLA-B (A), HLA-DR (B), HLA-A (C) and HLA-DQ (D). The amplicons are 1090 bp, 280 bp, 909 bp and 275 bp, respectively. Primers were purchased from Lifecodes Corp, Stamford, CT.

ABI sequencing of the HLA-A Locus





RELEVANCE

Patients with CUC are at an increased risk for developing CRC. The carcinogenesis of this CUC/CRC presents diagnostic challenges that are unique to this patient population. This is demonstrated by our preliminary data both in the lack of concordance between slide diagnostics and chart review (18.8% of all cases reviewed to date) and by the finding that 10 patients (20%) had a recent biopsy (≤ 2 years before the cancer diagnosis) that contained no observable precancerous changes. Therefore, there remains a need to elucidate markers that will identify patients at greatest risk for developing CRC so that alternative treatments or testing strategies can be found. A **DNA marker** such as HLA or TNF- α offers distinct advantages over other diagnostics in that it can be performed whether the disease is quiescent or active, can be done using blood, and can be utilized to incorporate overall family testing strategies.

The inclusion of **protein markers** in our test population will also give insights into disease etiology that may offer new avenues of therapy. There are trials currently underway for TNF- α monoclonal antibody therapy, but there still is a need to correlate polymorphisms of this gene with response to therapy and with in situ expression.

The **database** of patient information, pathology results and results from this study as well as the stored DNA will serve as a long term source for conducting future studies into CUC. By also investigating demographic, familial and patient social characteristics, we hope to elucidate other disease mechanisms that may play a role in overall CUC pathogenesis.

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