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L'Institut de recherche Terry Fox

Surgical margins: Assessment using molecular and phenotypic alterations associated with risk in a trial of fluorescence-guided surgery -- **the COOLS trial**

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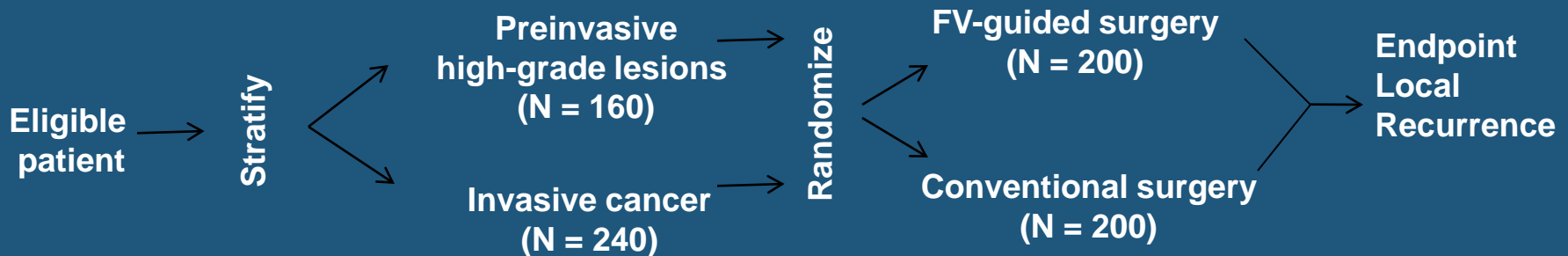
Problem of Oral Cancer; Promise of FV?

- Oral cancer a major global health problem
- 5-year survival = 30-60%
- **High rate of tumor recurrence (10-30%)**
- Diseased tissue often extends beyond clinically visible tumor margins
- New handheld light, fluorescence visualization (FV)
 - Preliminary data: reduces recurrence ~ 7 fold



CANADIAN OPTICALLY-GUIDED APPROACH FOR ORAL LESIONS SURGICAL (COOLS) TRIAL

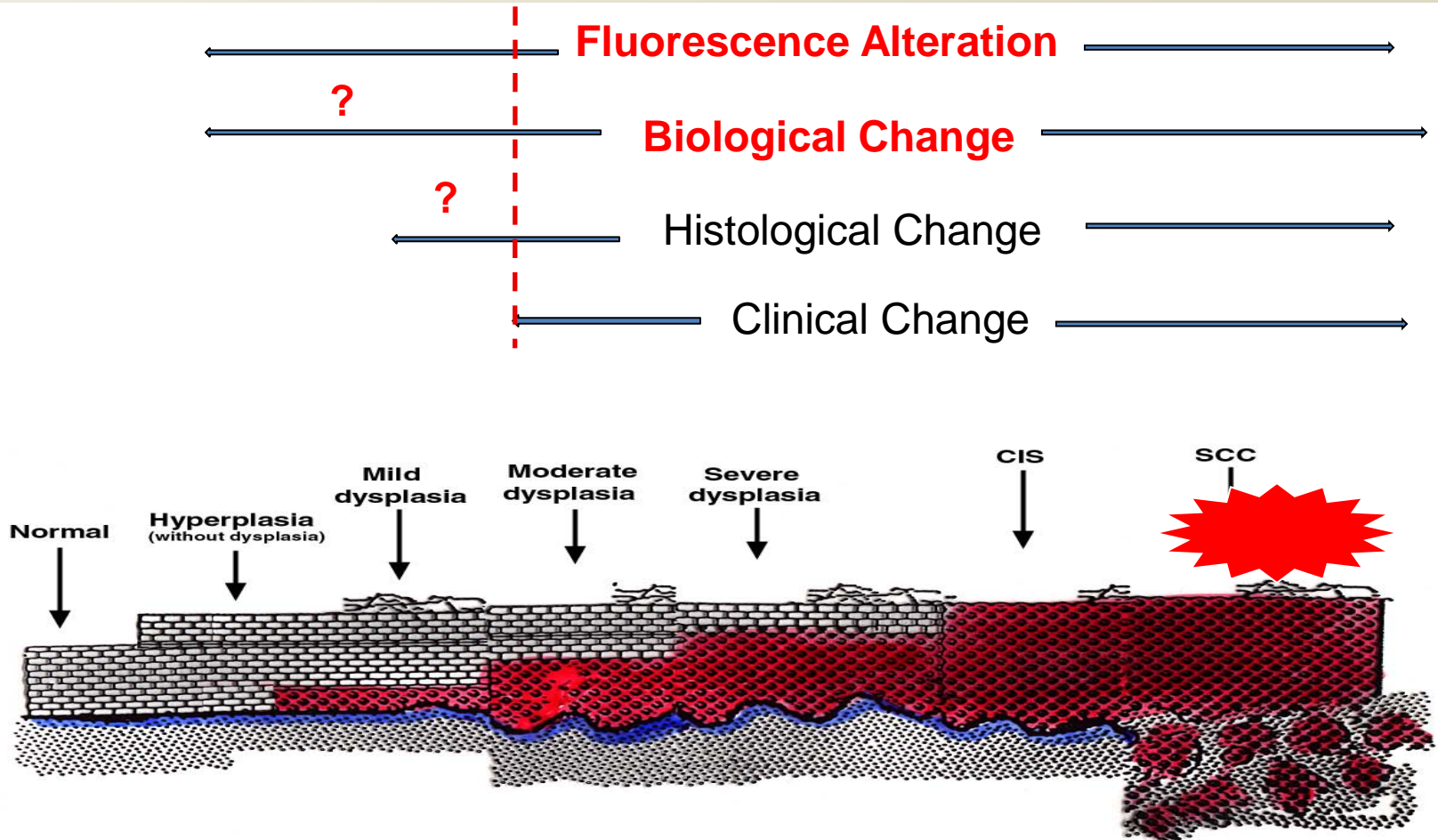
- 5 year, phase III national multi-centric study (9 sites)
- Goal: To determine the efficacy of using fluorescence visualization (FV)-guided surgery to reduce local recurrence of oral cancer



4 components:

1. Clinical – Scott Durham, Catherine Poh
2. Scientific – Issues, Questions, Approaches, Pilot work
3. Health Economics – Ian Cromwell
4. Knowledge Translation – Sarah Gustin

ISSUE WE ARE EXPLORING – IDENTIFICATION OF HIGH-RISK FIELDS



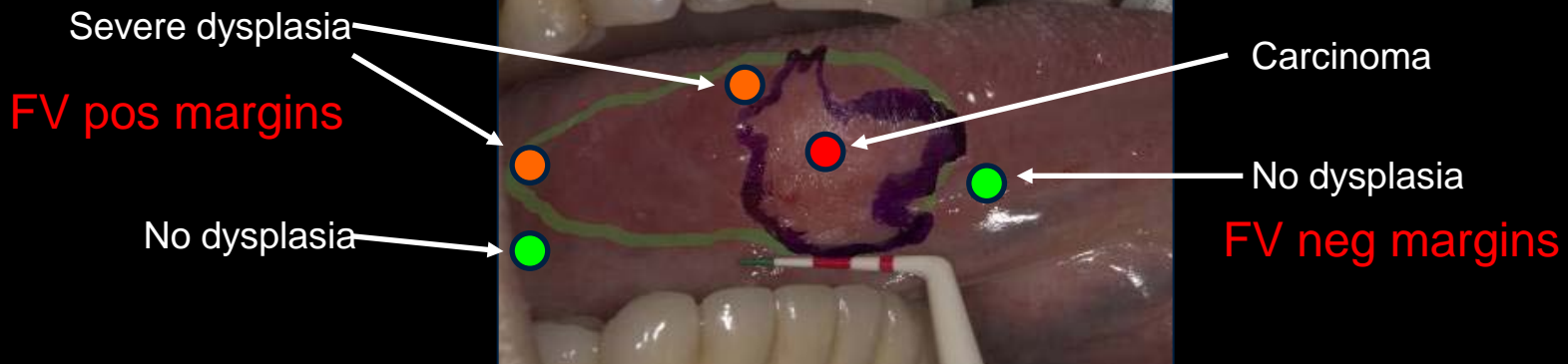
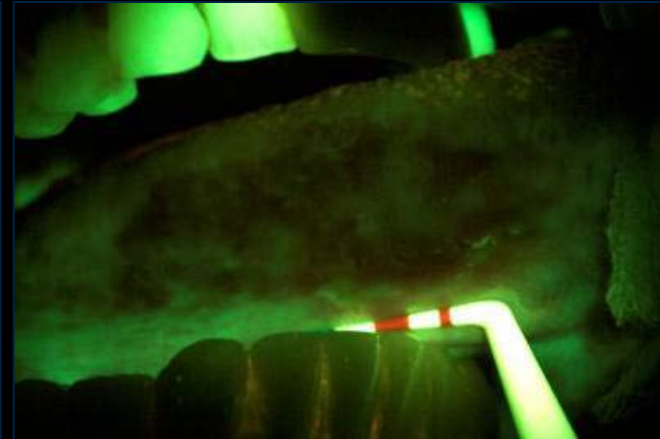
Spread of genetically altered cells across the oral mucosa
Sometimes clinically apparent...sometimes histologically...altered behavior/risk



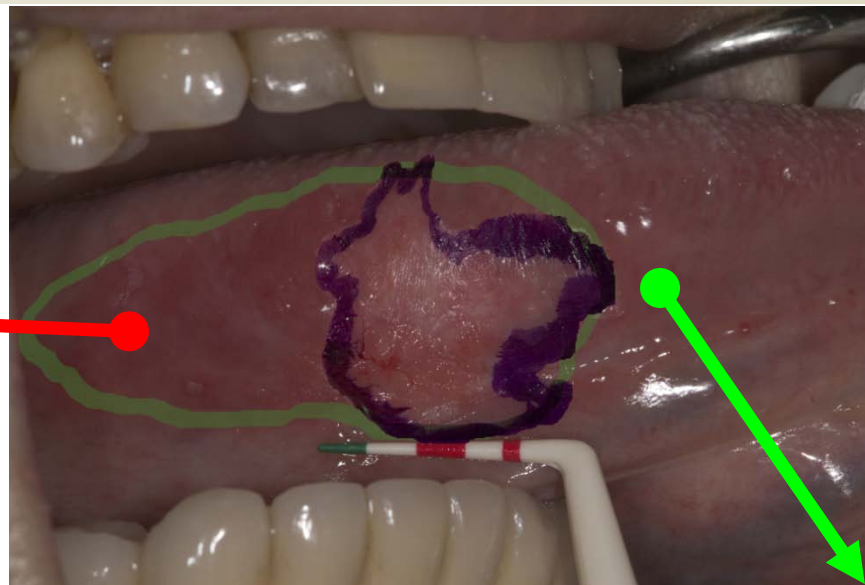
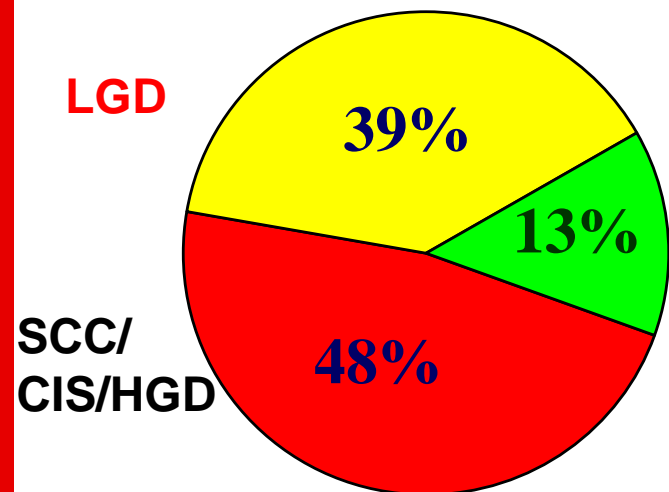
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Orientation to study: FV positive / FV negative margins

19/20 lesions has FV positive area outside the tumor area; the FV margin is asymmetric relative to the tumor



EARLY DATA SUPPORTING TRIAL FV MARGIN STATUS & HISTOLOGY



FVpos margin (n=38)

SCC/CIS/HGD = 18 (48%) ●

LGD = 15 (39%) ●

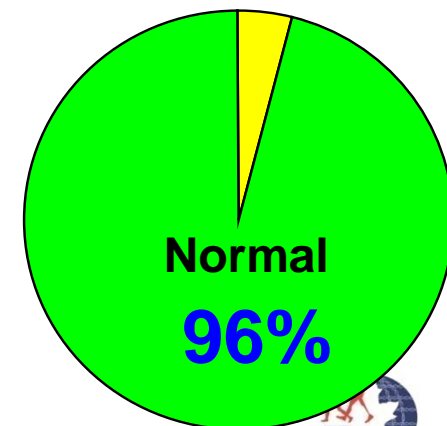
No dysplasia = 5 (13%) ●

FVneg margin (n=74)

Ca/CIS//HGD = 0 ●

LGD = 3 (4%) ●

No dysplasia = 71 (96%) ●



QUESTION

- Will the **shift of surgical field** that occurs with use of fluorescence visualization (FV) improve capture of **high-risk tissue** in occult margins?
 - Histological risk: severe dysplasia and higher
 - What about low-grade margins or “normal” margins? If FVpos will they still be high-risk?



METHODS

- Focusing on surgical margins histologically diagnosed as low-grade dysplasia or “normal”
- Assess for progression risk, using 2 approaches:
 - Loss of heterozygosity (LOH) hotspot analysis – **molecular** indicator of clones
 - Quantitative pathology (QP) – **phenotypic** indicator of change to nuclear organization
- Determine whether Fvpos margins have greater frequency of high-risk markers (compared to Fvneg)



LOH Hotspot analysis

- Requires little DNA
- Works with DNA from archival tissue
- **Good indicator of high-risk clones**
- **Strong association with risk of progression**
 - Multiple labs: Sidransky/Califano, Mao/Lippman, Partridge (Retrospective analysis; robust technique)
 - In use for ongoing chemoprevention trials
- **Developed retrospectively with archival samples and validated prospectively in ongoing Oral Cancer Prediction Longitudinal Study (Total of 400 patients)**

LOH Hotspot Analysis

■ Retrospective cohort (116 patients)

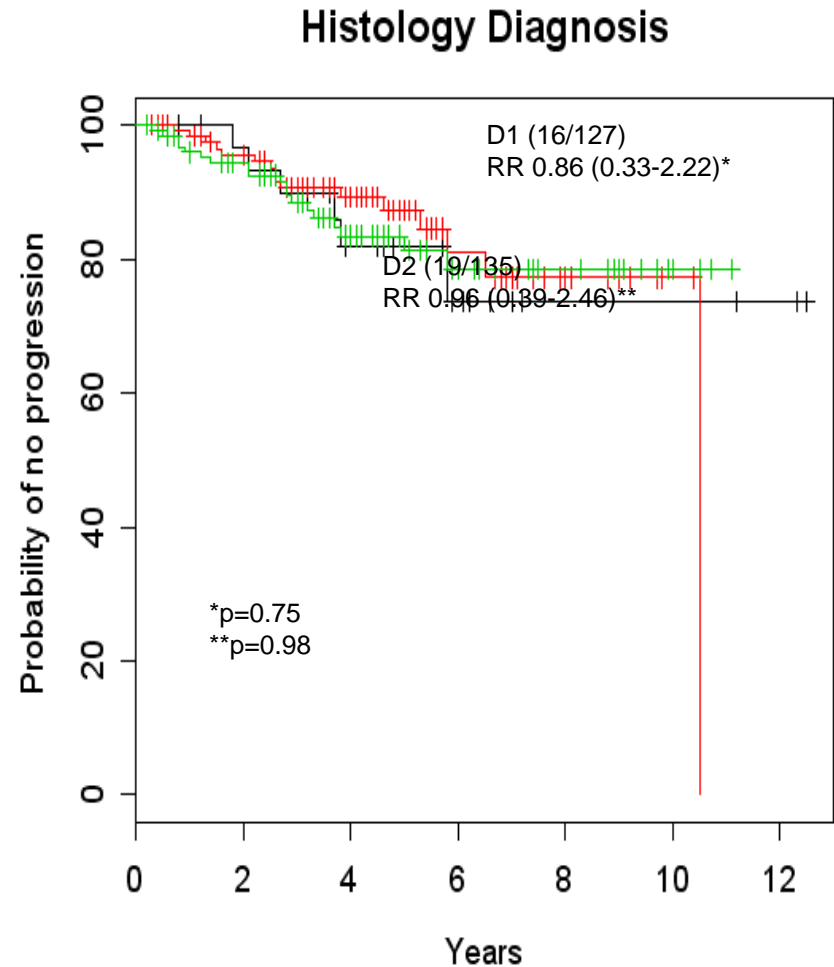
- Hyperplasia, mild or moderate dysplasia
- Follow-up: 43.5 (36.0 - 103.3) months. (Median, 25th and 75th percentiles)
- 29 (25.0%) progressed
- Found: LOH patterns that predicted progression

■ Prospective cohort (296 patients)

- Enrolled 1997 to 2007
- Follow-up: 44.6 (29.3 - 63.9) months
- 41 (13.9%) progressed to severe dys & higher
- Confirmed: Previous LOH patterns
- New predictors: Hotspots on 3 arms: 9p, 4q, 17p
- Confirmed with retrospective cohort

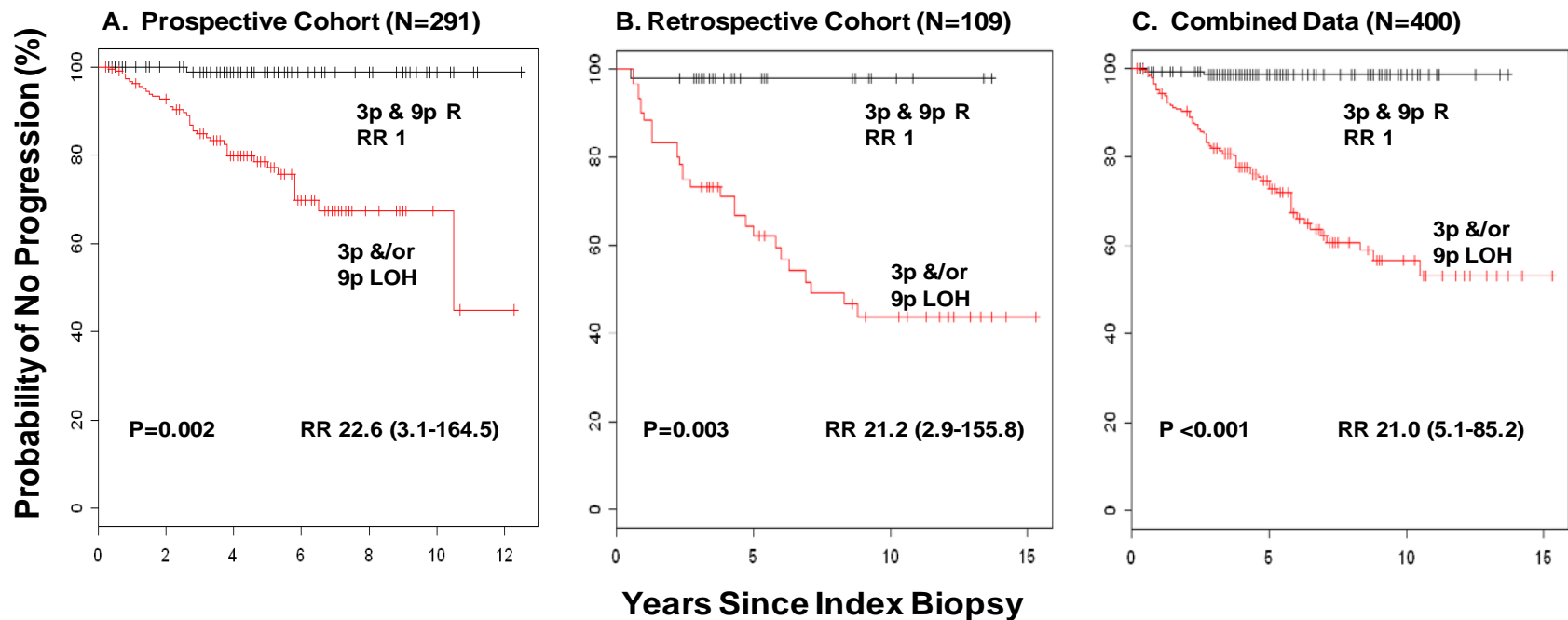
HISTOLOGY

Histology does not distinguish lesions that will progress from those that will not progress

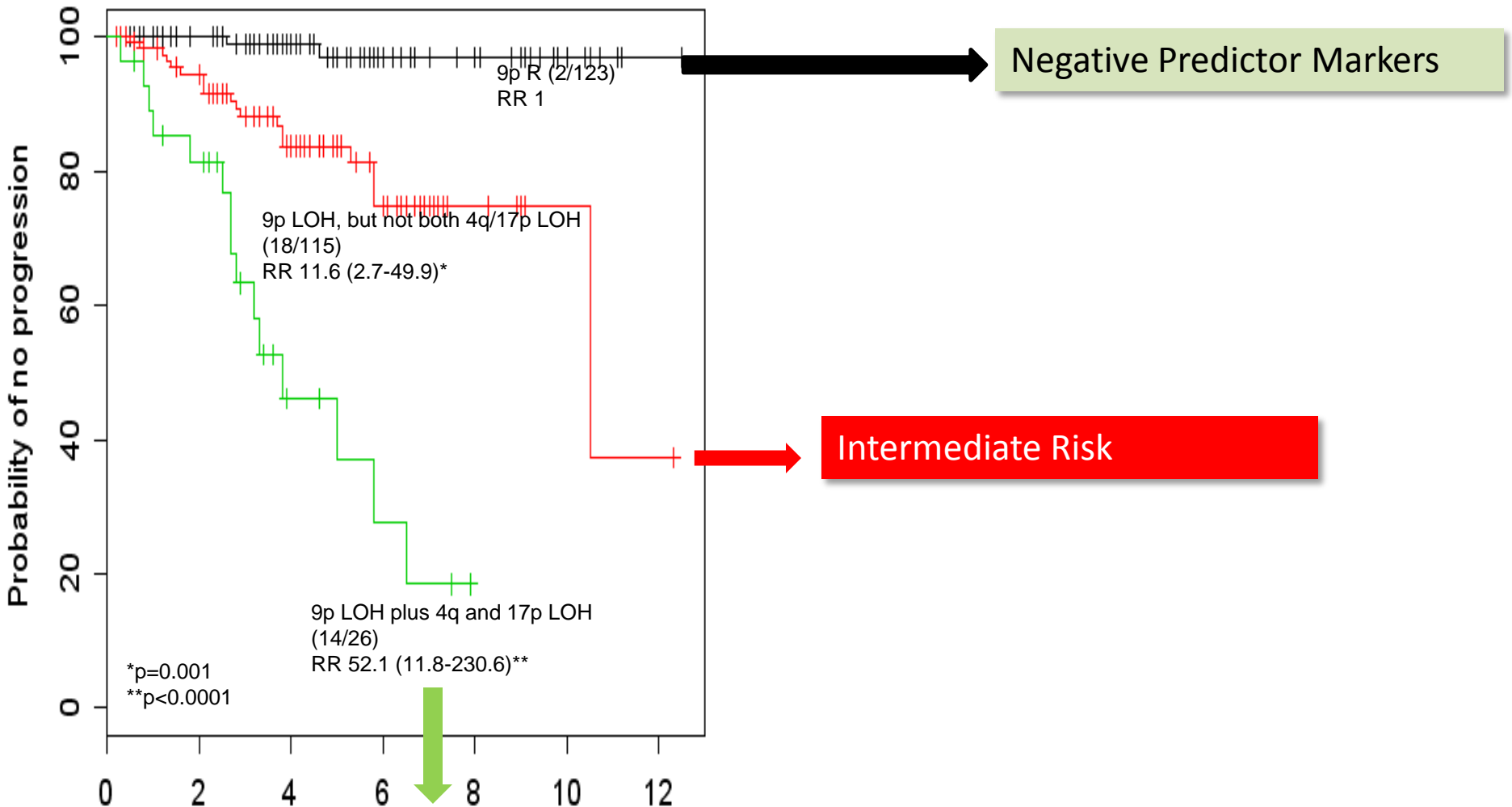


LOH HOTSPOT ANALYSIS VALIDATION

- Only 1% of lesions with low-risk pattern progressed = strong negative predictor
- High-risk pattern present in 97.5% of progressing cases
- 22.6-fold increase in progression risk



Additional Markers to detect HR clones

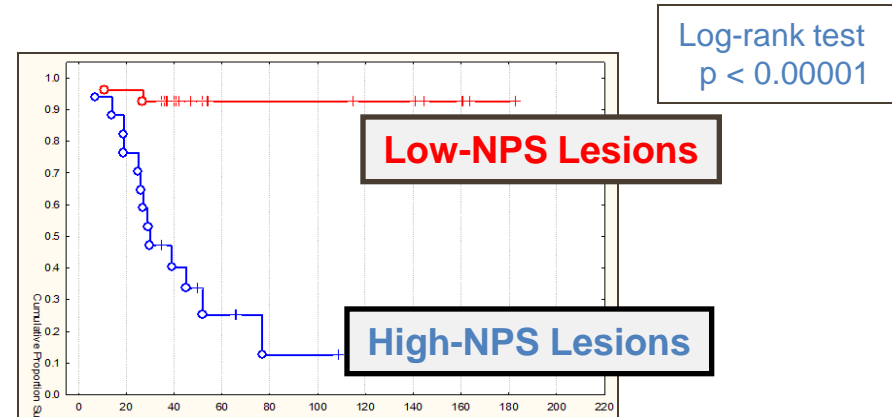
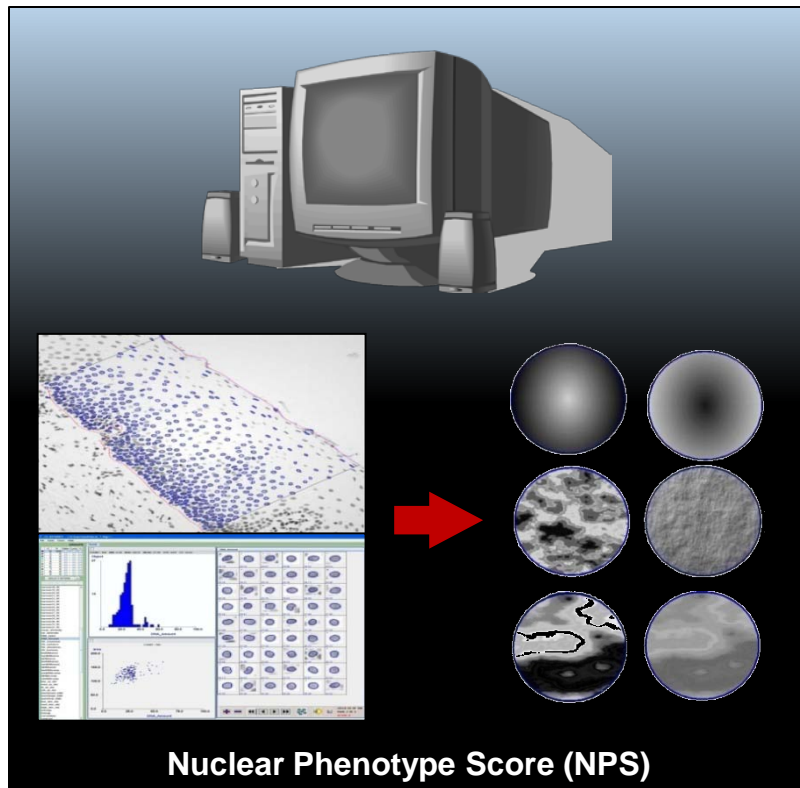


High Risk of progression
5- yr progression rate = 63.1% (~2/3)

Note : Severe dysplasia
5yr progression = 70%
BC treats.

QUANTITATIVE PATHOLOGY

- Textural consequences of molecular/epigenetic change
- Detects subtle phenotypic alteration associated with progression risk
- Algorithm measures DNA distribution - chromatin features – altered nuclear organization
- Development retrospective cohort - Validated in OCPL prospective cohort



Nuclear Phenotype Score Determination by QP

- Elevated NPS is associated with 9.9-fold increase in risk of progression
- Also associated with high-risk LOH molecular pattern

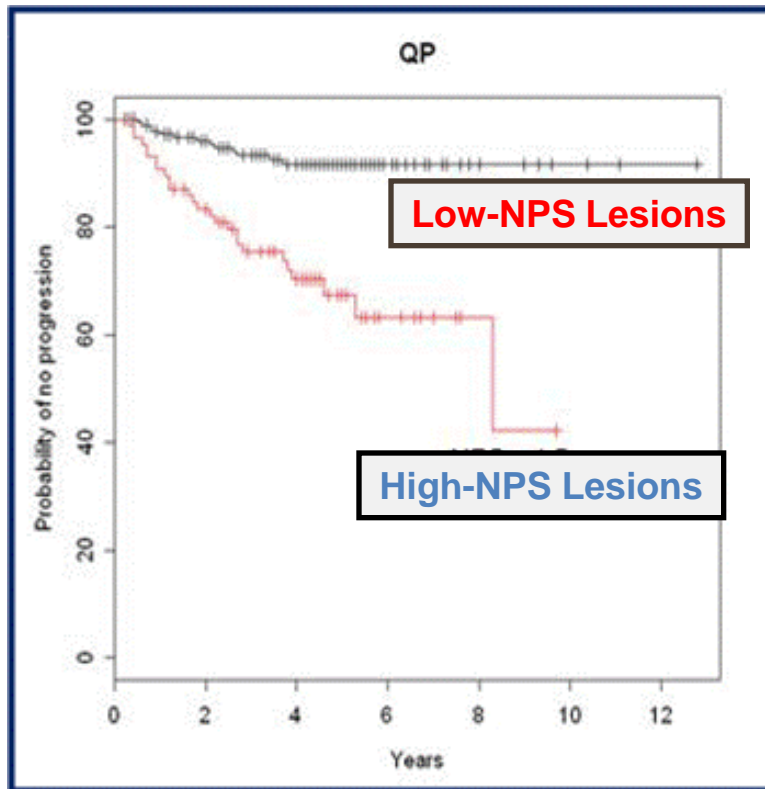
Cancer Res 2008; 68: (9): 3099



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VALIDATION

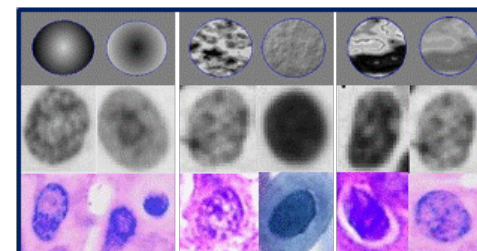
- LGOPLs biopsies from 284 patients were studied: 47 hyperplasias, 116 mild and 121 moderate dysplasias.
- The images were analyzed using the NPS (a combination of five nuclear morphometric features that best distinguish 'normal' nuclei from 'abnormal' nuclei for each sample)



Nuclear Phenotype Score Determination by QP

- Elevated NPS is associated with significant increase in risk of progression

Cox Proportional Hazards Models on Time to Progression		
Variable	HR (95% CI)	P Value
NPS	4.5 (2.3-8.8)	<0.0001
Mild Dysplasia	1.1 (0.4-2.8)	0.9
Moderate Dysplasia	1.6 (0.6-3.9)	0.33



HYPOTHESES

Looking at margins that are low-grade or histologically normal:

- Hypothesis 1: High-risk molecular clones and QP changes will be more frequently present in FVpos compared to FVneg margins
- Hypothesis 2: If cases with a higher frequency of molecular clones and phenotypic changes in margins more likely to have recurrence



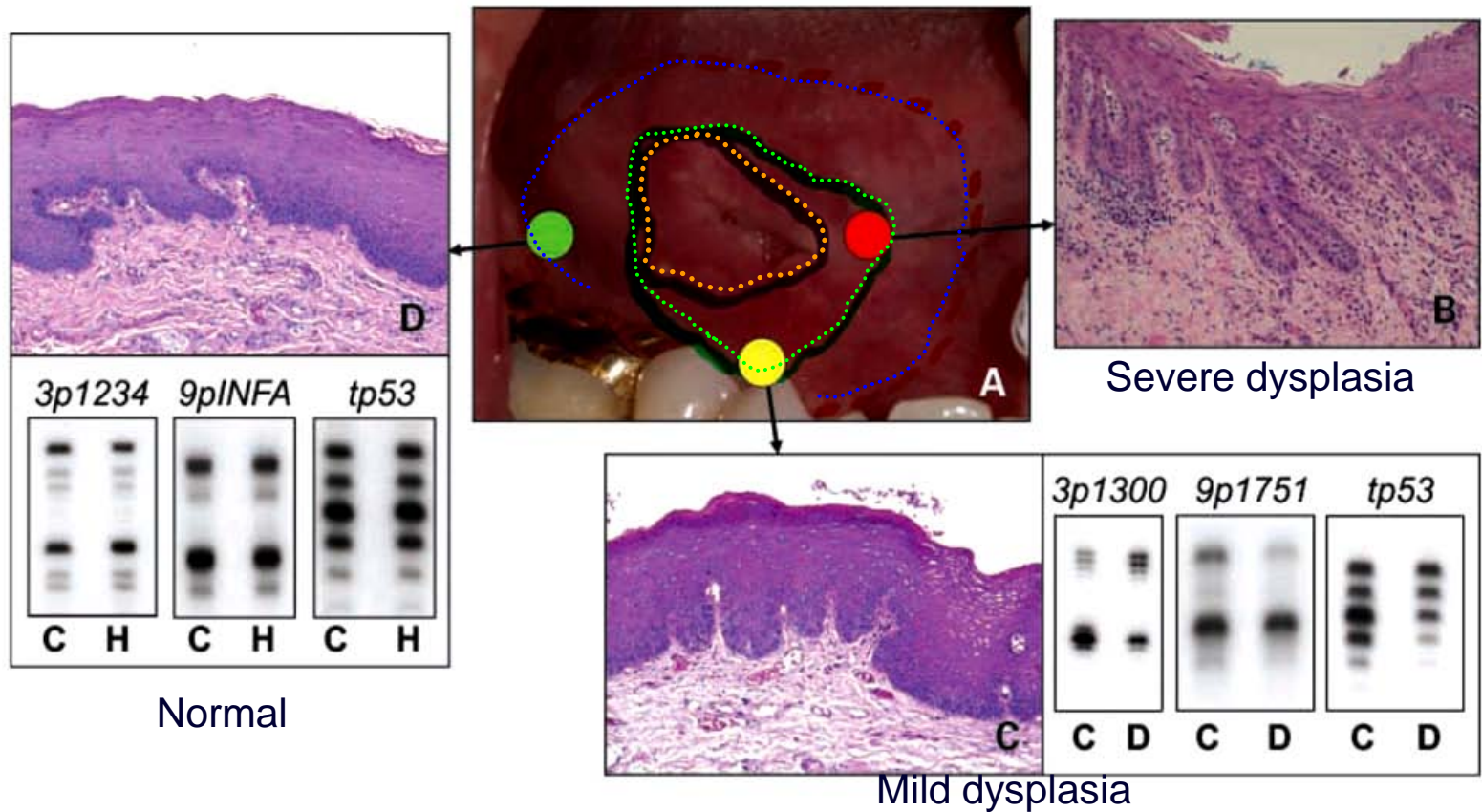
Concept: Shift in surgical field

“Undercut versus overcut”

..... Visible Margins

..... FV Margins

..... Excision Margins

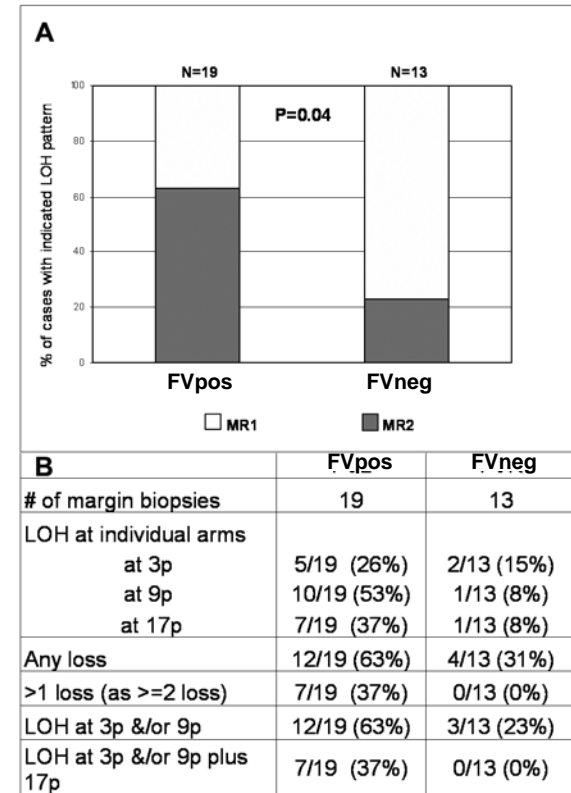
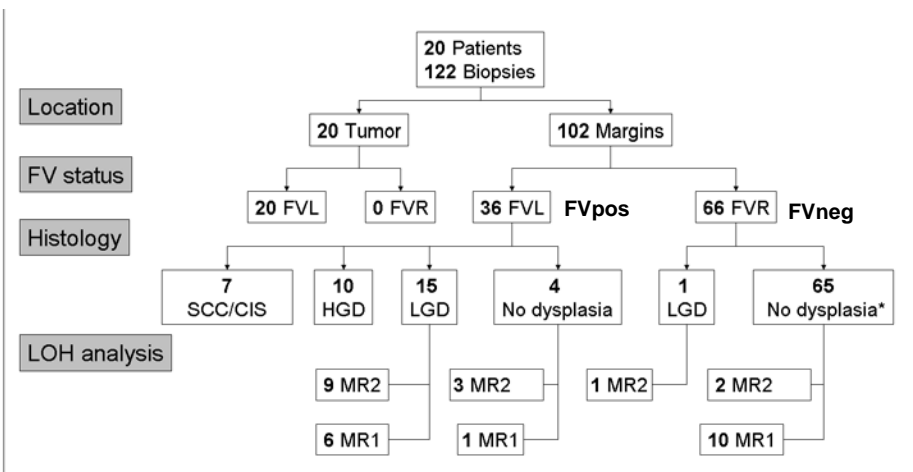


Spare normal tissue???

Catch High-Risk Tissue?????

PILOT DATA ON SURGICAL MARGINS

- High-risk LOH pattern significantly elevated in FV positive margins compared with FVneg margins (63% versus 23%, $P = 0.04$).
- Loss of autofluorescence (FVpos) are associated with presence of high-risk molecular clones in clinically occult margins



MR1, LR = No LOH at 3p and 9p
MR2, HR = LOH at 3p and/or 9p



UNIQUENESS OF STUDY

- **Opportunity to learn about biology of disease: validating 2 promising markers for association with FV and with recurrence**
- **Unique specimens for future marker assessments: clinically occult, but Fvpos margins; surgical margins free of clinical or optical change, all with known outcome**
- **Unique opportunity of assessing width of tissue margin needed for clearance – e.g., how close to FV edge**



CONCLUSION

- Recent validation efforts suggest that the choice of LOH and QP are appropriate for margin evaluation.
- Markers will provide very useful information about the shift in fields associated with FV.
- Such consideration is critical to balance the benefit and risk of altering surgical margin delineation





ACKNOWLEDGEMENT

COOLS

Canadian Optically Guided Approach for
Oral Lesions Surgical Trial / Approche
canadienne d'optique guidée pour essai en
intervention chirurgicale des lésions orales

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