



Use of DNA repair Pathway analysis to predict overall survival in head and neck cancer patients treated with FHX based chemoradiotherapy.

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Abstract (updated)

Background: Concurrent chemoradiotherapy is a standard-of-care for head and neck cancer. Radiotherapy and chemotherapeutic agents damage DNA; lack of adequate repair induces tumor cell death. The network of DNA repair pathways was examined to predict FHX chemoradiotherapy outcomes.

Methods: Tissue specimens (paraffin) from 53 HNC patients were evaluated from tissue microarrays. Samples originated from patient groups from phase I/II studies: 1) poor-prognosis radiation-naïve, 2) re-irradiation. All were treated with FHX-based chemoradiotherapy. Ten DNA repair biomarkers XPF, pMK2, PAR, pH2AX, FANCD2, ATM, BRCA1, RAD51, ERCC1 (clone 8F1), and p53 were explored using IHC, automated image processing, and machine reading (>500 cells/read), for nuclear and cytoplasmic quantity, area, and intensity, and correlated with clinical outcome.

Results: There was low inter-core variability per tumor with median patient ranking signal to noise ratio of 15.0 across all markers. Patients were stratified into short and long survival groups by determination of critical marker thresholds. Five DNA repair biomarkers (RAD51, ATM, BRCA1, XPF, FANCD2) exhibited univariate significance for overall survival ($p = 2.65e-3$, $1.53e-2$, $4.77e-4$, $8.57e-5$, $1.32e-3$) (additional clinical parameters multivariate analysis was not feasible due to sample size). ERCC1 was not statistically significant ($p = 1.0$). Pair-wise biomarker combinations improved survival group discrimination. Combination of 4 DNA repair biomarkers (FANCD2, BRCA1, ATM, XPF) was also significant ($p = 1.31e-4$). Discriminant analysis demonstrated higher fractions of correctly identified patients in good/poor survival groups from four-marker tests.

Conclusions: Five DNA repair biomarkers predicted overall survival following FHX-based chemoradiotherapy. By contrast ERCC1 (8F1) was not significant. Combination of markers improved predictive ability in this study. The analysis of DNA repair pathways, particularly in homologous recombination, DNA damage response, and nucleotide excision repair, may be clinically useful. Validation in a larger, homogeneous patient population, and using platinum-based chemoradiotherapy, is indicated and currently ongoing.

Study Goals

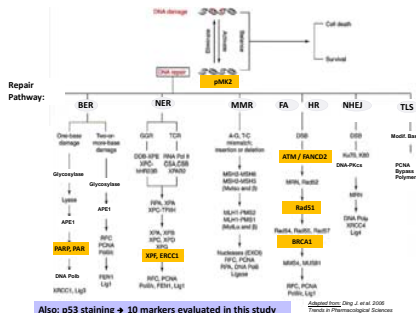
→ Screening of all major DNA repair pathways in parallel

- Identification of suitable markers
- Identification of suitable antibodies for FFPE tissue
- Optimization of staining and antibody validation
- Optimization of machine based scoring (Aperio) and comparison with pathologist scoring (tQC)

Study Question: Are DNA repair pathways important in recognizing responsiveness to chemoradiotherapy?

(previous work on induction chemotherapy presented at ASCO 2008)

DNA Repair - Background

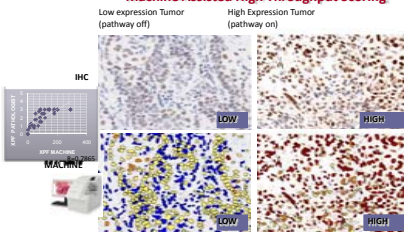


Also: p53 staining → 10 markers evaluated in this study

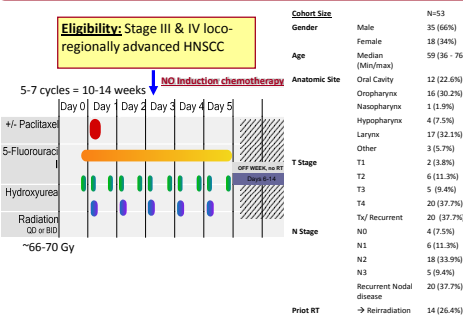
Methods

- N = 53 patients with 3 core primary biopsy specimens on a Tissue Microarray (TMA)
- N = 48 patients with Overall Survival (OS)
- N = 43 patients evaluable for Disease-Specific Survival (DSS) and Time to Progression (TTP)
- TMA methods are conducted by antibody-based IHC, digital pathology, and image analysis
- Evaluation of Multimarker Models to improve predictive power

Machine Assisted High Throughput Scoring



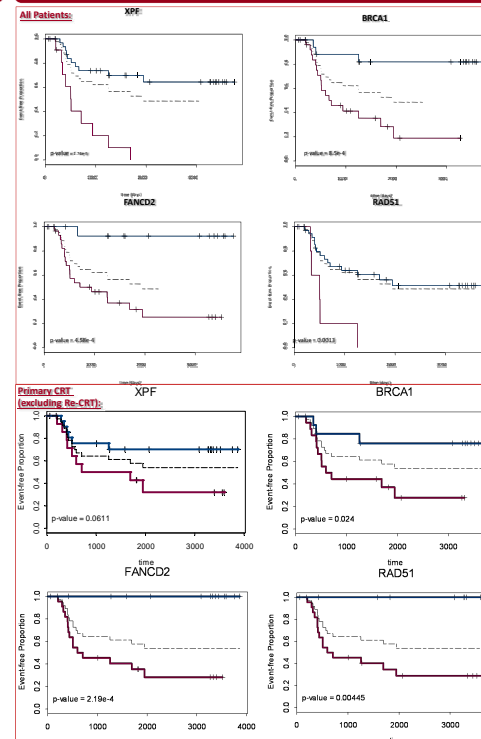
Treatment / Patient Population



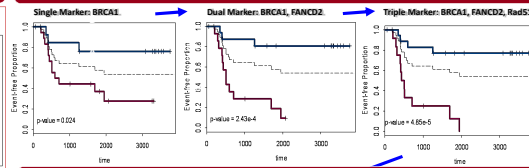
RESULTS: Univariate Analysis Disease Specific Survival

MARKER	P val	AUC	AER	Sens	Spec	Pos Pow	Neg Pow	HIGH RISK	LOW RISK
FANCD2	2.19E-04	0.83	0.22	1.00	0.64	0.65	1.00	26	15
RAD51	4.45E-03	0.70	0.25	1.00	0.53	0.65	1.00	26	10
BRCA1	0.02	0.74	0.27	0.80	0.67	0.67	0.80	19	18
XPF	6.11E-02	0.61	0.32	0.60	0.73	0.60	0.73	16	25
p53	2.33E-02	0.58	0.34	1.00	0.35	0.58	1.00	29	7
ATM	6.27E-02	0.55	0.38	0.53	0.71	0.62	0.63	15	21
pMK2.C	1.86E-01	0.51	0.38	0.20	0.95	0.75	0.60	4	34
PAR	1.02E-01	0.61	0.43	1.00	0.29	0.48	1.00	33	6
pMK2.N	4.18E-01	0.56	0.44	0.53	0.58	0.50	0.61	18	20
pH2AX	1.19E-01	0.57	0.46	1.00	0.20	0.48	1.00	34	5
ERCC1	5.36E-01	0.53	0.47	0.80	0.29	0.50	0.63	27	9

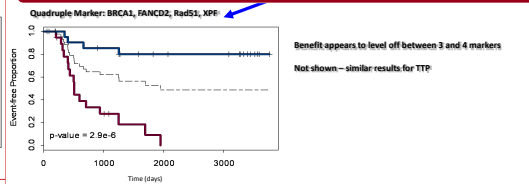
RESULTS: Single Marker Analysis – Disease Specific Survival



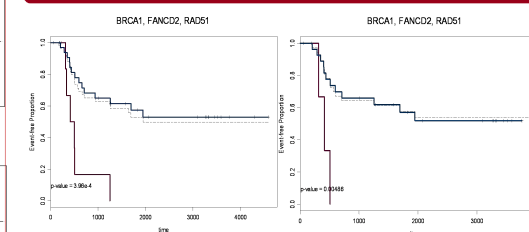
RESULTS: Single versus Multiple Marker Model (DSS)



RESULTS: Four Marker Model – Disease Specific Survival



RESULTS: Three Marker Analysis – Overall Survival



Caveats / Weaknesses

- Caveats:**
 - Exploratory analysis in a single cohort
 - Small sample number
 - Marker thresholds (high/low groups) optimized for this cohort (possible bias?)
 - Mix of reirradiation patients (small sample number)
 - Unable to separate RT from Chemo effect
 - Unclear if FHX based results apply to Cisplatin based CRT

- Strengths:**
 - Consistency across multiple markers with unequivocal separation of curves
 - Consistency across multiple outcomes (OS, DSS, TTP (not shown))
 - Consistent results for entire cohort and in primary radiation subset
 - Replication of the developed algorithm in a 3rd cohort of reirradiation patients is starting (→ potential large for predictive biomarker)

Conclusions

- DNA repair markers may be able to predict survival in patients with FHX based chemotherapy
- Several biomarkers in the Homologous Recombination pathway are identified as beneficial – specifically: *Rad51*, *FANCD2*, *BRCA1*, *ATM*
- XPF (Nucleotide Excision Repair) is predictive
- “Synergy” between DNA repair biomarkers: 3- and 4-marker models perform best in this pilot cohort

- Future Steps:**
 - Replication of the developed algorithm in a 2nd independent chemoradiation cohort is ongoing (validation cohort)
 - Replication of the developed algorithm in a 3rd cohort of reirradiation patients is starting (→ potential large for predictive biomarker)