Pharmacogenetics of Upper Aerodigestive Cancers:
A Journey from hope to despair and back

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Upper Aerodigestive Tract Cancers

- Head and Neck Cancer
- Esophageal Cancer
- Lung Cancer

- “similar” epidemiological risk factors (smoking)
- “similar” therapies (cisplatin, taxane)
- “similar” markers (EGFR)
Goals of Presentation

• Define Genetic Variation and Pharmacogenetics

• Present a historical perspective on development of this field in upper aerodigestive cancers

• Present opportunities for current and future research in this area
Human Variation

• Each human person carries millions of normal variations in our DNA
  – Variations dictate everything from hair colour to shape of toenails
  – Common variations are called polymorphisms
  – We carry the same variations throughout life except when a mistake is made during cell division, where errors may lead to diseases such as cancer
  – Each parent passes $\frac{1}{2}$ of our variations to our children
Genetic Polymorphism

• Common variations in genetic code (>1% incidence in study population)
  – Otherwise called germline mutations
  – Inherited
  – Can be determined from a blood sample
Single Nucleotide Polymorphisms

- A Single substitution in the DNA sequence
  - A → C
  - A → T
  - A → G
Insertion (Deletion)

- **C A T C A T C A T C A T C A T C A T C A T C A T**
  - **His** **His** **His** **His** **His** **His** **His** **His**

- **C A T C A T C A T C A T A C A T C A T C A T C A T**
  - **His** **His** **His** **Thr** **Ser** **Ser** **Ser** **Ser**
VNTR (Variable Number Tandem Repeats)
Microsatellite
Copy Number Variants

• A duplication or deletion involving > 1kb of DNA
  – Non-homologous end joining
  – Non allelic homologous recombination
  – Can affect expression levels, function
Polymorphisms can alter function through multiple mechanisms:

- Conformational change
- Binding site change
- Early termination
Polymorphisms can alter function through multiple mechanisms

- Promoter
- Exon
- Intron
- UTRs
- Regions that are spliced into non-coding RNAs
- “junk areas”
Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

PRO-DRUG

ACTIVE DRUG

INACTIVE DRUG
Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**Diagram:**
- Drug absorption
- PRO-DRUG
- ACTIVE DRUG
- INACTIVE DRUG
Pharmacogenetics

• Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

Drug absorption

PRO-DRUG ➔ Metabolic enzymes ➔ ACTIVE DRUG ➔ INACTIVE DRUG
Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**Diagram:**
- **Drug absorption**
- **PRO-DRUG**
- **Metabolic enzymes**
- **ACTIVE DRUG**
- **INACTIVE DRUG**

**Drug**
- transporters
- binders
**Body habitus**
Pharmacogenetics

• Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

PRO-DRUG

Drug absorption

Metabolic enzymes

ACTIVE DRUG

Drug - transporters
- binders

Body habitus

INACTIVE DRUG

Drug targets
Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

Diagram:
- Drug absorption
- PRO-DRUG
- Metabolic enzymes
- ACTIVE DRUG
- Drug targets
- INACTIVE DRUG
- Drug excretion

- Drug
  - transporters
  - binders
- Body habitus
History of “Successes” in Pharmacogenetics

Genes involved in PK
Drug Absorption/Transport
Activation/Metabolism/Excretion

Genes involved in PD
Drug mechanism of action.
targets/downstream effectors

Hematology/Oncology Drugs with FDA label modifications

<table>
<thead>
<tr>
<th>Drug</th>
<th>Genetic Variation</th>
<th>Involved in:</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MP and AZA</td>
<td>TPMT</td>
<td>PK</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>UGT1A1</td>
<td>PK</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Warfarin</td>
<td>CYP2C9 &amp; VKORC1</td>
<td>PK and PD</td>
<td>Toxicity</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>CYP2D6</td>
<td>PK</td>
<td>Efficacy</td>
</tr>
</tbody>
</table>
UGT1A1 gene and irinotecan: TA indel polymorphism is associated with ANC nadir

Correlation between absolute neutrophil count (ANC) nadir (log scale) and TA indel genotype
1. Candidate polymorphism approach

- Data supporting association with disease, outcome, or function
  - Biologic (genotype-phenotype, \textit{in vivo} studies)
  - \textit{In silico} “predictive function”
  - Evolutionary
  - Epidemiologic
Platinum-DNA adduct formation

Cisplatin
XPD and XRCC1 polymorphisms

• Differential activity
• Case-control studies of lung cancer risk

Can these polymorphisms explain differences in outcome after platinum treatment in NSCLC patients?
Hypothesis

- Variant genotypes of the DNA repair genes, *XPD* and *XRCC1*, affect survival in advanced NSCLC patients treated with platinum-based regimens
Effect of DNA repair on outcome

DNA Repair

- Removal of platinum-DNA adducts
- Function of platinum chemotherapy
- Survival

- Number of somatic mutations
- Tumor aggressiveness
- Survival
Patient selection

251 patients with histologically-proven advanced NSCLC, 5+ years follow-up and available medical records

112 patients treated with platinum agents at MGH Cancer Center

103 patients with complete genotype data for XPD and XRCC1
Genotyping

- DNA from whole blood
- Genotyping by PCR-RFLP
  - XPD/ERCC2 (Asp312Asn)
  - XRCC1 (Arg399Gln)
Clinical Outcome

• Overall survival
• Dates of death confirmed through
  – SSDI
  – Outpatient/inpatient records
  – MGH tumor registry
• Patients not deceased were censored at
  – Last date of clinic follow-up or
  – Last date known alive
## Patient Characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>103</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>26 (25%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>30 (29%)</td>
</tr>
<tr>
<td>IV</td>
<td>47 (46%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53 (51%)</td>
</tr>
<tr>
<td>Female</td>
<td>50 (49%)</td>
</tr>
<tr>
<td>No. of events</td>
<td>86</td>
</tr>
<tr>
<td>Median age</td>
<td>58 (32-77)</td>
</tr>
</tbody>
</table>

Gurubhagavatula et al, JCO 2005
# Median Survival Times

<table>
<thead>
<tr>
<th>By Stage</th>
<th>n</th>
<th>MST (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>103</td>
<td>14.9</td>
</tr>
<tr>
<td>By Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>26</td>
<td>28.6</td>
</tr>
<tr>
<td>IIIB</td>
<td>30</td>
<td>16.0</td>
</tr>
<tr>
<td>IV</td>
<td>47</td>
<td>9.3</td>
</tr>
</tbody>
</table>

**Median f/u time**

| Median f/u time | 63.9 months |

Stage was not associated with any genotypes

Gurubhagavatula et al, JCO 2005
**XRCC1 variant genotypes are associated with poorer survival**

<table>
<thead>
<tr>
<th>Genetic polymorphism</th>
<th>n</th>
<th>MST (mos)</th>
<th>Logrank test</th>
<th>Hazard Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>XRCC1 Arg399Gln</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg** (Wildtype)</td>
<td>51</td>
<td>17.3</td>
<td>p=0.07</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Arg/Gln (Hetero)</td>
<td>42</td>
<td>11.4</td>
<td></td>
<td>1.45 (1.03-2.05)</td>
</tr>
<tr>
<td>Gln/Gln (Variant)</td>
<td>10</td>
<td>7.7</td>
<td></td>
<td>2.11 (1.49-2.98)</td>
</tr>
</tbody>
</table>

*by Cox proportional hazards model adjusted for stage and PS  
**homozygous wildtype

Gurubhagavatula et al, JCO 2005
Survival Probability

Survival (months)

Log rank p=0.07

XRCC1 Genotypes and Overall Survival

Gurubhagavatula et al, JCO 2005
### XPD variant genotypes are associated with poorer survival

<table>
<thead>
<tr>
<th>Genetic polymorphism</th>
<th>n</th>
<th>MST (mos)</th>
<th>Logrank test</th>
<th>Hazard Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPD Asp312Asn</td>
<td></td>
<td></td>
<td>p=0.003</td>
<td></td>
</tr>
<tr>
<td>Asp/Asp** (Wildtype)</td>
<td>50</td>
<td>16.3</td>
<td></td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Asp/Asn (Hetero)</td>
<td>41</td>
<td>15.2</td>
<td></td>
<td>1.36 (0.97-1.90)</td>
</tr>
<tr>
<td>Asn/Asn (Variant)</td>
<td>12</td>
<td>6.6</td>
<td></td>
<td>1.84 (1.31-2.58)</td>
</tr>
</tbody>
</table>

*by Cox proportional hazards model adjusted for stage and PS
**homozygous wildtype

Gurubhagavatula et al, JCO 2005
Survival Probability vs. Survival (months)

Log rank p=0.003

Gurubhagavatula et al, JCO 2005
The combination of variant genotypes is associated with poorer survival

<table>
<thead>
<tr>
<th>Genetic polymorphism</th>
<th>n</th>
<th>MST (mos)</th>
<th>Logrank test</th>
<th>Hazard Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td>p=0.009</td>
<td></td>
</tr>
<tr>
<td>0 variants**</td>
<td>26</td>
<td>20.4</td>
<td></td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>1 variant allele</td>
<td>40</td>
<td>16.6</td>
<td></td>
<td>1.41 (1.11-1.80)</td>
</tr>
<tr>
<td>2 variant alleles</td>
<td>24</td>
<td>11.0</td>
<td></td>
<td>1.99 (1.56-2.53)</td>
</tr>
<tr>
<td>3 variant alleles</td>
<td>13</td>
<td>6.8</td>
<td></td>
<td>2.80 (2.20-3.57)</td>
</tr>
</tbody>
</table>

*by Cox proportional hazards model adjusted for stage and PS
**double homozygous wildtype

Gurubhagavatula et al, JCO 2005
Number of \textit{XPD/XRCC1} Variant Alleles and OS

Log rank $p=0.009$

Gurubhagavatula et al, JCO 2005
Summary

- Evaluation of DNA repair gene polymorphisms is feasible
- *XPD* and *XRCC1* variant genotypes, both alone and in combination, are associated with decreased overall survival in platinum-treated NSCLC patients
- …then information surfaced on the importance of *ERCC1* in cisplatin-related DNA repair
Kaplan-Meier curves of the *ERCC1 C8092A* polymorphism (P=0.006, by logrank test)

Zhou et al, CCR 2005
DNA Repair Polymorphism and Grade III/IV Gastrointestinal (GI) Toxicity

- 147 NSCLC patients treated first-line with combined chest radiation and platinum-based chemotherapy
- 93% were PS ECOG 0/1
- Stage
  - 6% were stage I and II, 46% were stage IIIA, 39% were stage IIIB, and 9% were stage IV
- Treatment
  - 42% received cisplatin, 58% received carboplatin
- Thirty-one (21%) patients experienced Grade III/IV GI toxicity (nausea, n=10; vomiting, n=4; esophagitis, n=20)

ASCO abstract, 2004
Zhou et al, CCR 2005
## ERCC1 polymorphism and GI toxicity

<table>
<thead>
<tr>
<th>ERCC1 C8092A</th>
<th>Total n</th>
<th>Grade III/IV GI toxicity: n (%)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All stages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>85</td>
<td>12 (14%)</td>
<td>1.00</td>
</tr>
<tr>
<td>C/A or A/A</td>
<td>62</td>
<td>19 (30%)</td>
<td>2.83 (1.24-6.47)</td>
</tr>
<tr>
<td><strong>Stage III only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>72</td>
<td>11 (15%)</td>
<td>1.00</td>
</tr>
<tr>
<td>C/A or A/A</td>
<td>53</td>
<td>17 (32%)</td>
<td>2.77 (1.15 – 6.66)</td>
</tr>
</tbody>
</table>

Zhou et al, CCR 2005
“Future Directions”

• Validation studies
  – Same disease site, same drug
  – Different disease site, same drug

• Other DNA repair gene polymorphisms
  – “Comprehensive” evaluations
Since then...lung cancer validation?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Study Details</th>
<th>SNP/Variant</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1</td>
<td>7 3 Asian/4 Cauc. (n= 36-248)</td>
<td>XRCC1Arg399Gln</td>
<td>Platinum. Gln/- associated with worse GI toxicity in single Asian study [AOR 2.53 (1.06-6.03); p=0.03]. Gln/Gln worse survival in stage IIIA/B in US study, better survival in Italian study.</td>
</tr>
<tr>
<td></td>
<td>3 2 Asian/1 USA (n=36-229)</td>
<td>XRCC1 Arg194Trp; XRCC1 Arg280His (single Asian study)</td>
<td>Arg/Arg worse toxicity with gem/docetaxel in single study (p=0.03). No tox. assoc. with cisplatin in 2nd study. No OS assoc.</td>
</tr>
<tr>
<td>XPD</td>
<td>7 2 Asian (n=36-248)</td>
<td>XPD Asp312Asn; XPD Lys751Gln</td>
<td>No assoc. in 5 studies. Variant genotype (-312Asn/Asn) worse OS in single study (p=0.003). -751Lys/Lys assoc. with Gr 4 neutropenia in one (p=0.02)</td>
</tr>
<tr>
<td>ERCC1</td>
<td>Count</td>
<td>Description</td>
<td>Results</td>
</tr>
<tr>
<td>---------------</td>
<td>-------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7 Asian; 4 Cauc. (n=65-423)</td>
<td>ERCC1 118C/T</td>
<td>Platinum. C/C better OS/RR in 3 Asian studies. No associations in 4 studies in Caucasians.</td>
<td></td>
</tr>
<tr>
<td>3 1 Asian; 2 USA (n=128-423)</td>
<td>ERCC1 8092C/A;</td>
<td>Platinum. C/C had better OS and A/- had increased GI toxicity in US study</td>
<td></td>
</tr>
<tr>
<td>1 China, n=162</td>
<td>ERCC1(262G/T;433T/C; 3525C/T; 4855C/T; 14443C/A)</td>
<td>Small cell only + Carboplatin/VP16. 262T/T worse OS [AHR 1.98; p=0.017].</td>
<td></td>
</tr>
</tbody>
</table>
Reasons for lack of validation

- Heterogeneous populations
- No clear functional genomics data
- Small sample sizes
- “Fuzzy” hypothesis
- Multiple hypotheses
- “not a true matching validation set”
Inherited Genetic Variation and Lung Cancer Outcomes

Horgan AM, Yang B, John T, Cescon D, Wheatley-Price P, Shepherd FA, Liu G.

- 237 genetic variations in 79 studies.
- Survival was the outcome in 89% of the studies
- Toxicity was outcome in 22%.
- Candidate polymorphisms in the DNA repair/synthesis pathway were the most frequently studied.
- Results were conflicting
- Many had little functional genomic data
- Strong evidence supporting validation in large-scale confirmatory studies of any single polymorphism was lacking.
- Heterogeneity in study populations and inconsistencies in methodology between studies were common.
- Almost all were candidate polymorphism-based
## Treatment Modalities

<table>
<thead>
<tr>
<th>Treatment Modalities</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation to Primary Tumor</td>
<td>66 (64%)</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; line Chemotherapy Regimens</td>
<td></td>
</tr>
<tr>
<td>Carboplatin-Taxane</td>
<td>63 (61%)</td>
</tr>
<tr>
<td>Cisplatin-Vinca Alkaloid</td>
<td>21 (20%)</td>
</tr>
<tr>
<td>Cisplatin-Etoposide</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Other Platinum Combinations</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Non-Platinum 1&lt;sup&gt;st&lt;/sup&gt; Line Agent</td>
<td>8 (8%)</td>
</tr>
</tbody>
</table>
DNA-Repair Gene Polymorphisms Predict Favorable Clinical Outcome Among Patients With Advanced Squamous Cell Carcinoma of the Head and Neck Treated With Cisplatin-Based Induction Chemotherapy

Miguel Quintela-Fandino, Ricardo Hitt, Pedro P. Medina, Soledad Gamarra, Luis Manso, Herman Cortes-Funes, and Montserrat Sanchez-Cespedes

A

Survival Probability

XPD751 Pol

XPD751 Com

P = .0012

Time to Death (months)

B

Survival Probability

XPD312 Pol

XPD312 Com

P = .0012

Time to Death (months)

C

Survival Probability

XRCC-1 Pol

XRCC-1 Com

P = .0044

Time to Death (months)

D

Survival Probability

ERCC1 Pol

ERCC1 Com

P = .80

Time to Death (months)

E

Survival Probability

5 Pol

6 Pol

7 Pol

P < 10^-4

Time to Death (months)

F

Survival Probability

1 or more Pol variants

0 Pol variants

P < 10^-4

Time to Death (months)
Reasons for discrepancy?

- Cisplatin vs carboplatin?
- Disease site specificity?

- False positive result(s)?
  - Small sizes
  - Heterogeneous populations (treatments)?

<table>
<thead>
<tr>
<th>Induction regimen*</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP + radiotherapy</td>
<td>26</td>
<td>25.2</td>
</tr>
<tr>
<td>CDDP + fluoropyrimidine</td>
<td>31</td>
<td>30.1</td>
</tr>
<tr>
<td>CDDP + fluoropyrimidine + taxane</td>
<td>42</td>
<td>40.8</td>
</tr>
<tr>
<td>Cisplatin + cetuximab</td>
<td>4</td>
<td>3.9</td>
</tr>
</tbody>
</table>
DNA repair polymorphisms and Cisplatin-based and non-cisplatin treated esophageal cancer patients

(Bradbury et al in preparation)
Possible Validation Datasets

- Lung Cancer: BR.10, BR.24, TORCH, BRC4
- Head and Neck: HN.6
- Esophageal: RTOG, TROG

- Local observational datasets:
  - Lung Cancer (>300 with cisplatin treatment)
  - Head and Neck (>100 with cisplatin treatment)
  - Esophageal cancer (>100 with cisplatin treatment)
Inherited Genetic Variation and Lung Cancer Outcomes
Horgan AM, Yang B, John T, Cescon D, Wheatley-Price P, Shepherd FA, Liu G.

• Best candidates (at least 2 positive studies, any number of negative underpowered studies allowed)
  – *ERCC1* 118C/T in Asians
  – *EGFR intron 1* and –216G/T in EGFR treated patients
  – *GSTM1-null*
  – *p53Arg72Pro*
  – *MDM2309*
Pharmacogenetic Example: EGFR polymorphisms and EGFR TKIs (2004-)

Review of existing PK/PD/PG data

SNP - HapMap

Proprietary PK data

PGRN and public source PK/PG/PD data

In silico and bioinformatic determination of best targets

Haploview/Tagger

I2D/PPI Networks

SIFT/PolyPhen/Coddle
Pharmacogenetic Example: EGFR polymorphisms and EGFR TKIs (2004-)

Promoter Analysis AMPL
Luciferase Promoter Assays

Gene Expression/Binding Assays
Collaboration with A. Adjei (Mayo/RPCI)/STTARR
Haplotype Constructs and functional Binding and Expression assays

Identification of key targets to test in patient samples

Liu et al, CR 2005
-216G/T polymorphism & PFS/OS

<table>
<thead>
<tr>
<th></th>
<th>T/-</th>
<th>G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>58(63%)</td>
<td>34(37%)</td>
</tr>
<tr>
<td>Med PFS</td>
<td>4.1 mos</td>
<td>2.1 mos</td>
</tr>
<tr>
<td>Adj. HR</td>
<td>0.62</td>
<td>reference</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.38-0.99)</td>
<td></td>
</tr>
</tbody>
</table>

Log rank p=0.005

Liu et al, TPJ 2007
| EGFR | 7 | 3 Asian, 4 Cauc  
n = 70-173 | EGFR intron 1  
(CA)$_n$Shorter/Longer | With Gefitinib. No assoc. with OS in 5 studies. Longer assoc. with worse OS in single Asian study ($p=0.039$)  
No Gefitinib: Longer associated with better OS in single US study ($p=0.03$) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Italy; n=124</td>
<td>ABCG2 421C/A</td>
</tr>
</tbody>
</table>
|      | 4 | 1 Asian; 3Cauc  
n = 92-170 | EGFR -216G/T  
EGFR -191C/A | Gefitinib: T allele of -216 better PFS alone or in combination with Intron 1S/S in US study. Combination assoc. with better OS ($p=0.02$). EGFR1 GC haplotype worse OS ($p=0.015$) but only when analysis restricted to stages 0 and 1 in 2nd study. |
Possible Validation Studies

EGFR TKI treated patients
• Lung Cancer
  – BR.21
  – BR.19
  – TORCH
  – BRC4
• Head and Neck Cancer
  – HN.6

Lung Cancer General prognosis (*GSTM1, p53, MDM209*)
• BR10+BR19 no treatment arms, BR24 both arms
• 24 polymorphisms had at least one positive association with outcomes in HNC
• All 24 and one since published are being validated in 540 early stage HNC patients all treated uniformly with radiation.
  – Using data/specimens from Phase III Secondary prevention study of AT/BC.
To the extreme → Exploratory candidate polymorphism array chip

- 520 esophageal cancers from Boston
  - All stages
  - All treatments

- 1536 Candidate polymorphisms in various cancer-related, oncogene, tumor suppressor, cell cycle, apoptotic, xenobiotic metabolism and pharmacogenetic pathways chosen from polymorphism literature of upper aerodigestive cancers

- Validation in Toronto samples +/- RTOG? +/- TROG?
2. Tagging Approach

- Tag/Block analysis
  - One SNP ≠ function
  - Utilizes LD structure to reduce number of polymorphisms required to be genotyped to identify most/all of the common genetic variation in a gene
    - Still need to pick the genes of interest
    - Potential misclassification since most haplotypes are inferred from computer programs
Haplotypes and Tagging

- Multiple SNPs located close together.
- Haplotype blocks are smallest segments of DNA containing SNPs that tend to be conserved without recombination and inherited as a unit.
- Haplotype analysis = analyze the block rather than a single SNP.
Reducing the number of markers
In Haplotypes
Using 5 TagSNPs to define variation in a gene

Each colour is a different haplotype block
In silico mapping human EGFR linkage disequilibrium within the area resequenced. Pairwise D' values are shown ($P < 0.05$).
Tagging

- Hard to do using archival FFPE samples (too much DNA)
- Exploratory

- Easier to do using blood
  - TORCH
  - BRC4/MARVEL
  - BR.24
TagSNPs

- TagSNP has association with outcome
- In silico functional evaluation of SNPs in LD with TagSNP
- Deep resequencing to identify new polymorphisms in LD → In silico functional evaluation
- Biological functional evaluation → often partnered with site known to have the constructs, etc. If not, will need to develop
  - Replicate/validate in other datasets
Combined TagSNP and candidate SNP selection approaches


(A) VEGF-2578 C/A
(B) VEGF-1154 G/A.
**Table 4. Relationship of VEGF Genotype With Grade 3 or 4 Hypertension**

<table>
<thead>
<tr>
<th>Single Nucleotide Polymorphism</th>
<th>Patients</th>
<th>% of Patients With Grade 3 or 4 Hypertension</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VEGF-634</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>27</td>
<td>15.3</td>
<td>.013</td>
</tr>
<tr>
<td>GC</td>
<td>82</td>
<td>46.3</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>68</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>CC v GC + GG</td>
<td></td>
<td></td>
<td>.005</td>
</tr>
<tr>
<td><strong>VEGF-1498</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>60</td>
<td>33.9</td>
<td>.056</td>
</tr>
<tr>
<td>CT</td>
<td>82</td>
<td>46.3</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>35</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>TT v CC + CT</td>
<td></td>
<td></td>
<td>.022</td>
</tr>
<tr>
<td><strong>VEGF-2578</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>36</td>
<td>20.8</td>
<td>.32</td>
</tr>
<tr>
<td>CA</td>
<td>72</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>65</td>
<td>37.6</td>
<td></td>
</tr>
<tr>
<td>CC v CA + AA</td>
<td></td>
<td></td>
<td>.16</td>
</tr>
<tr>
<td><strong>VEGF-1154</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>15</td>
<td>9.4</td>
<td>.29</td>
</tr>
<tr>
<td>GA</td>
<td>54</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>91</td>
<td>56.9</td>
<td></td>
</tr>
<tr>
<td>GG v GA + AA</td>
<td></td>
<td></td>
<td>.15</td>
</tr>
</tbody>
</table>

Abbreviation: VEGF, vascular endothelial growth factor.
VEGFR TKI Pathway Approaches

VEGFR TKI

CYP3A4?

CYP2D6?

Metabolites?

VEGFA

Cell membrane

KDR/VEGFR2

phospholipase C-gamma (PLCG2)

protein kinase C (PRKCB1)

RAF1

MEK1/2 and ERK1/2

(MAPK3, MAPK1, MAP2K1, MAP2K2)

Multiple Cell signalling pathways

FLT1/VEGFR1
Polymorphisms of KDR Gene Are Associated With Coronary Heart Disease

Yibo Wang, PhD,* Yi Zheng, MD,* Weili Zhang, PhD,* Hui Yu, MS,* Kejia Lou,* Yu Zhang,* Qin Qin, MD,† Bingrang Zhao, MD,† Ying Yang, MD,‡ Rutai Hui, MD, PhD*

Beijing, Tianjin, and Shandong, People’s Republic of China

**Figure 1** SNP604C-Bearing KDR Promoter Had Lower Transcription Activity

The pGL3 luciferase reporter contained either the T (pGL3-T) or C allele (pGL3-C) at the promoter -604 locus. Values represent the average of 6 experiments and the bars represent the standard deviation. The pGL3-Basic was used as a negative control without any promoter sequence, and pGL3-control was used as a positive control. KDR = kinase insert domain-containing receptor; SNP = single nucleotide polymorphism.

**Figure 2** SNP1102 and SNP1713 Influenced the Binding Efficiency of VEGF to KDR

HEK293A cells were transfected with 8 μg of pDNA2.1-LCNeo (KDR-A, KDR-H, KDR-VQ, or KDR-VH). After 36 h, the cells were rinsed with cold phosphate-buffered saline 3 times, and the binding of vascular endothelial growth factor (VEGF165; 10 ng/ml, R&D Systems, Minneapolis, Minnesota) was carried out in binding buffer containing CMEM, 25 mmol/L HEPES (pH 7.4), 1 μg/ml trypsin, and 0.1% gelatin for 2 h at 4°C. Then the cells were rinsed with cold phosphate-buffered saline 5 times, lysed with cell lysis buffer, followed by enzyme-linked immunosorbent assay. The values were the ratio of VEGF to KDR. The experiments were repeated 3 times, and 2 replicates were performed for each experiment. The values were presented as means, and the bars represented standard deviations. Abbreviations as in Figure 1.

**Figure 3** Serum Levels of KDR Antigen Were Correlated With Genotypes of SNP604

Levels of the serum KDR were presented as means, and the bars represented standard deviations. The correlation was significant (p = 0.013), and Spearman coefficient for the existing correlation was r = -0.274. Nineteen samples with TT genotype, 14 with TC genotype, and 10 with CC genotype were analyzed. Abbreviations as in Figure 1.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Drug</th>
<th>Literature polymorphism</th>
<th>Number of tagSNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VEGF(A)</strong></td>
<td>VEGF TKI</td>
<td>-2578C&gt;A; -1498C&gt;T; -1154G&gt;A; -634G&gt;C; -460C&gt;T; +405G&gt;C; +936C&gt;T</td>
<td>20</td>
</tr>
<tr>
<td><strong>KDR/VEG FR2</strong></td>
<td>VEGF TKI</td>
<td>-604C&gt;T; +4422 AC repeat; V297I; Q472H</td>
<td>24</td>
</tr>
<tr>
<td><strong>FLT1/VEG FR1</strong></td>
<td>VEGF TKI</td>
<td>C519T (GenBank D64016)</td>
<td>41</td>
</tr>
</tbody>
</table>
Pathway analyses

- “Global pathway”
- Equally weighted pathways
- Weighted pathways
3. Genome-wide approach

- Microarray “Chip” technology
- Non-hypothesis driven
- Hypothesis generation
- Multiple comparisons – potential false positive associations
  - Costly
  - Needs multiple replications/validations in other datasets
  - Developmental bioinformatics and high dimensionality biostatistics required (techniques in development currently)
Head and Neck Cancer Radiation Outcomes Study (co-PIs Liu/Meyer)

- 540 HN cancers from Quebec in completed Phase III study of secondary prevention using alpha-tocopherol/beta-carotene
- DNA extracted/mature clinical outcomes data
- Toronto observational dataset validation?
- HN.6 validation?
Toronto Lung Cancer GWAS dataset
(PI – Hung/co-PI Liu)

- 419 Caucasians with Lung Cancer GWAS
- All stages and treatments
- CCO survival data
- Anne Horgan working on outcomes

• BR.24 validation?
• Boston validation?
• PMH validation?
Cutpoints

P-value

Location
Cutpoints

P-value vs. Location

- Scatter plot showing the relationship between P-value and Location.
Cutpoints

P-value

Location
Using networks and expression data to rank candidates
To Bioinformatically-inform the weighting of data

Cutpoints
Pharmacogenetic Epidemiology of Vitamin D in Head and Neck Cancer Outcomes

Vit D Resequencing data (A. Adjei, Roswell Park CI)

Bioinformatics analysis (I. Jurisica, PMH and S. Savas, Memorial)

Samples, Serum, and Epidemiology (F. Meyer, I Bairati, P. Douville, Laval)

Genotyping, Epidemiology and Analysis (G. Liu, W. Xu, PMH)

Multi-institutional, International Collaboration using Global, Unweighted and Weighted Pathway Analyses of Combined Candidate Polymorphism + Tagging Approaches + Secondary analysis of GWAS
Summary

- Traditional Candidate polymorphism selection requires
  - Rigorous functional genomic evaluations
  - Multiple validation datasets
- Pathway and Tagging Approaches may be more helpful
- GWAS has both potential benefits but limitations
- Bioinformatically informed analysis
Main Laboratory
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- Dave Cescon
- Jessica Hopkins

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- David Hedley
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- Ron Feld
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- Tom Waddell
- Shaf Keshavjee + many more

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