Assessing Genomic Sequencing Information for Health Care Decision Making: A Workshop

Shashikant Kulkarni, M.S (Medicine)., Ph.D., FACMG
Associate Professor of Pathology & Immunology
Associate Professor of Pediatrics and Genetics
Director of Cytogenomics and Molecular Pathology

GENOMICS AND PATHOLOGY SERVICES
GPS@WUSTL
CLINICAL TESTING • RESEARCH • EDUCATION

Washington University Physicians
Washington University School of Medicine in St. Louis

Children's HOSPITAL • ST. LOUIS

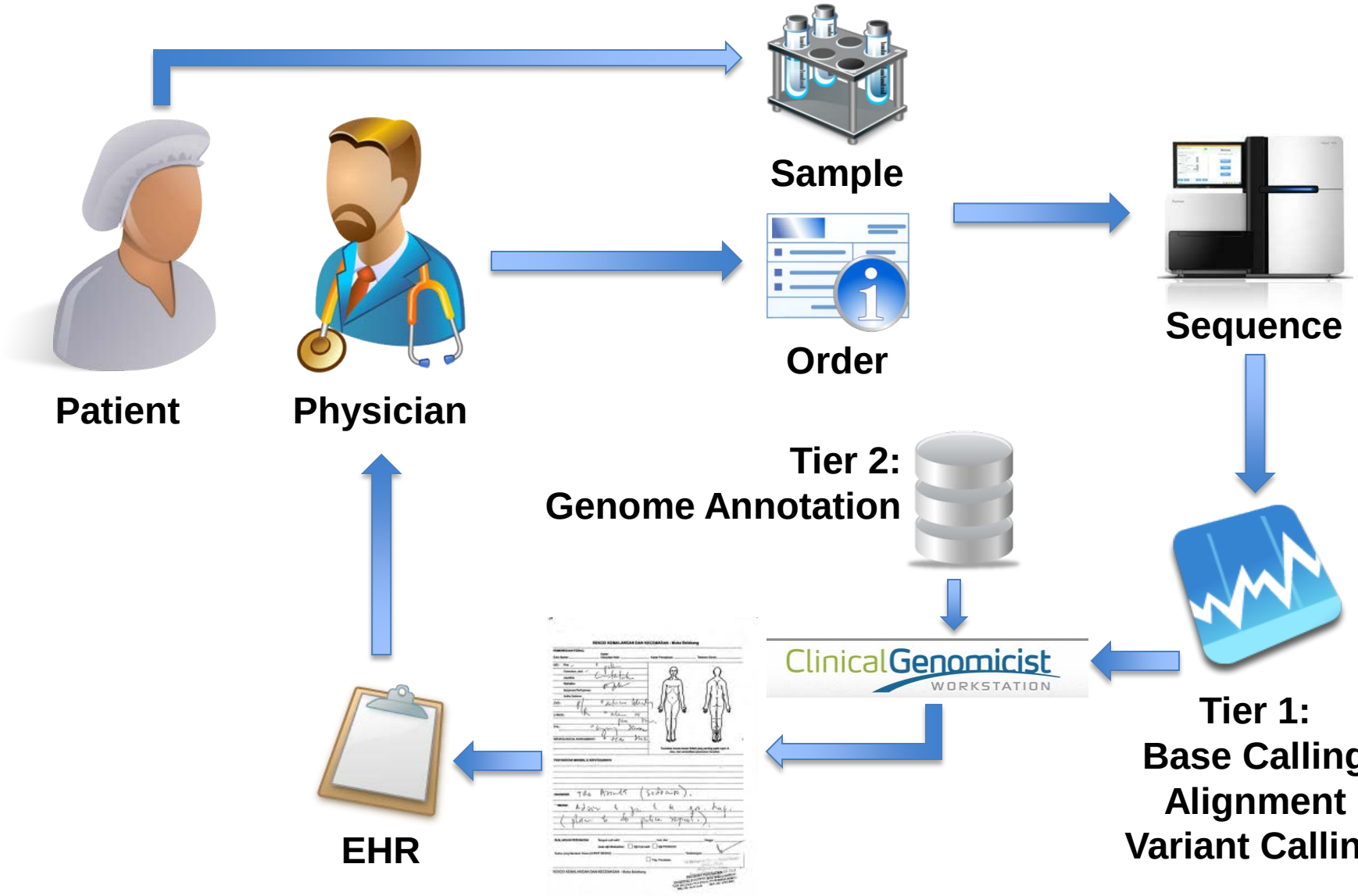
BARNES-JEWISH Hospital
BJC HealthCare

SITEMAN CANCER CENTER
BARNES-JEWISH HOSPITAL • WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
A National Cancer Institute Comprehensive Cancer Center

Evidence-based review of variants

Introduction to Workflow and process

NGS testing workflow (Clinical Genomicist workstation- CGW)

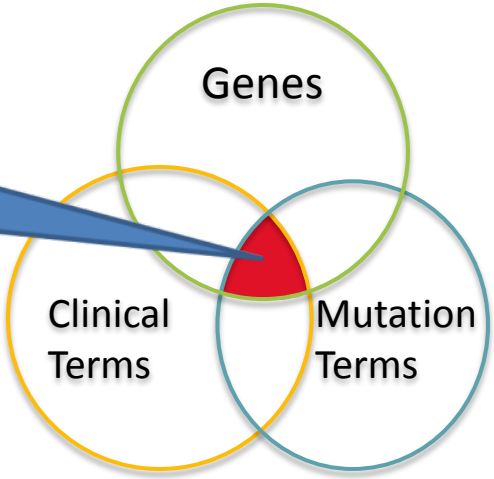


Critical assessment of variants

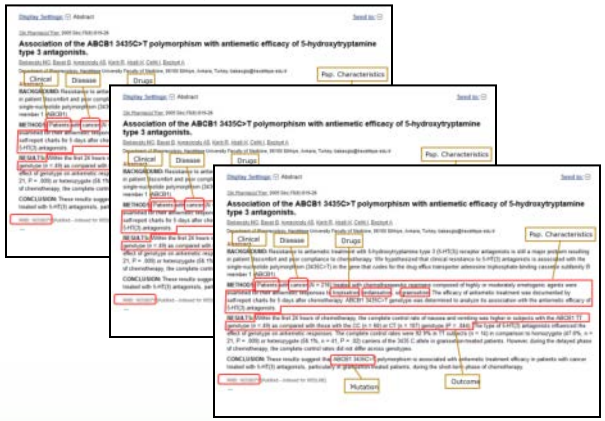
Variant analysis and filtration

Automated variant searches and selection process

Automated Searches



n=8261



Prefiltering

Inclusion Criteria n=1519 (25%)

Annotation Worthy n << 1519

My Allele	Nucleotide Change	Amino Acid Change	Disease	Drug (start with	Population characteristics	Evidence	Protein	ADME Observation	Patient Outcome (No Effect, Nothing	Evidence for patient	Variant	Reference
ABCB1.3;ABCB1.7	T1236>C; T3435>C	G412G; I1145I	Cancer	Imatinib	This study explored the association of 14	clinical data		Patients with the ABCB1			Biologically relevant	21311410
ABCB1.3;ABCB1.4;ABCB1.5;ABCB	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	We investigated the MDR1 T1236C, G	clinical data			Resistance was higher for patients		Biologically relevant	20204543
ABCB1.3	T1236>C	G412G	Cancer	Imatinib	Imatinib has been reported to be a	clinical data		Among the patients			Biologically relevant	18524988
ABCB1.3;ABCB1.5	T2677>G; T2677>A	S893A; S893T	Cancer	Imatinib	Imatinib has been reported to be a	clinical data			For the 2677G>T/A polymorphism, the		Biologically relevant	18524988
ABCB1.3;ABCB1.5;ABCB1.7	T1236>C;T2677>G;T2677>A	G412G;S893A; S893T	Cancer	Imatinib	Imatinib has been reported to be a	clinical data			1236C–2677G–3435C was statistically linked		Biologically relevant	18524988
ABCB1.7	T3435>C	I1145I	Cancer	Imatinib	The association between seven	clinical data			The CC genotype at the rs1045642 (C3435T)		Biologically relevant	21185600
ABCB1.4;ABCB1.5	T2677>G; T2677>A	S893A; S893T	Cancer	Imatinib	The association between seven	clinical data			The CC genotype at the rs1045642 (C3435T)			
ABCB1.3;ABCB1.4;ABCB1.5;ABCB	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	The aim of this study was to explore the	clinical data		This reduction in Imatinib		ABCB1 genotype		
ABCB1.3;ABCB1.4;ABCB1.5;ABCB	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	To investigate the relationship of ABCB1	clinical data			The ABCB1 1236CT/2677GT/3435CT			
ABCB1.3;ABCB1.7	T1236>C;T3435>C	G412G;I1145I	Cancer	Imatinib	NOT SIGNIFICANT: The aim of this study	clinical data			ABCB1 polymorphism was not significantly			
ABCB1.3;ABCB1.4;ABCB1.5;ABCB	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	NOT SIGNIFICANT: In this study,	clinical data			By multivariate regression analysis, only			
ABCB1.3;ABCB1.4;ABCB1.5;ABCB	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	NOT SIGNIFICANT: We investigated the	clinical data			ABCB1 polymorphism was not significantly			

Annotation

Interpretation

In chronic myeloid leukemia, patients treated with Imatinib who harbored the T1236>C; T2677>G; T2677>A; T3435>C genotype fared ….

Gene Abstracts Index

4527 EGFR (4527)
3087 KRAS (3087)
2626 TP53 (2626)
2240 BRCA1 (2240)
1717 BRCA2 (1717)
1591 KIT (1591)
1481 BRAF (1481)
1382 ERBB2 (1382)
1185 RET (1185)
1166 APC (1166)
1084 PTEN (1084)
1080 CDKN2A (1080)
1043 MLH1 (1043)
956 MYC (956)
942 PIK3CA (942)
899 FLT3 (899)
881 MET (881)
731 ATM (731)
717 CTNNB1 (717)
655 MAPK1 (655)

603 NRAS (603)
584 GSTP1 (584)
552 ABCB1 (552)
531 MTOR (531)
503 TAS2R38 (503)
497 VHL (497)
482 JAK2 (482)
471 ESR1 (471)
393 RUNX1 (393)
374 NPM1 (374)
354 SRC (354)
352 PDGFRA (352)
351 WT1 (351)
323 CYP2D6 (323)
315 ABL1 (315)
308 NF1 (308)
304 FBXW7 (304)
300 MLL (300)
298 FGFR1 (298)
294 ALK (294)

292 AKT1 (292)
283 CYP19A1 (283)
273 RB1 (273)
243 UGT1A1 (243)
243 HRAS (243)
233 IDH1 (233)
220 DPYD (220)
206 IL2RA (206)
205 CDKN2B (205)
204 CEBPA (204)
186 SMAD4 (186)
177 KDR (177)
176 PDGFRB (176)
154 CDH1 (154)
147 NOTCH1 (147)
143 FGFR3 (143)
131 PTCH1 (131)
130 CDA (130)
122 ESR2 (122)
120 IDH2 (120)

114 STK11 (114)
104 FGFR2 (104)
102 CYP2C19 (102)
99 PTPN11 (99)
98 ERG (98)
86 SMARCB1 (86)
85 TET2 (85)
84 SHH (84)
82 CYP2C9 (82)
82 ABCC2 (82)
81 MPL (81)
78 ERBB3 (78)
77 CSF1R (77)
76 RARA (76)
70 CYP2A6 (70)
59 GNAS (59)
53 FGFR4 (53)
53 DNMT3A (53)
52 FLT4 (52)
51 EZH2 (51)

49 SMO (49)
46 JAK1 (46)
46 ASXL1 (46)
45 MYD88 (45)
39 JAK3 (39)
38 MAP2K2 (38)
38 ERBB4 (38)
36 SLCO1B1 (36)
35 MAP3K1 (35)
33 FLT1 (33)
33 CYP2B6 (33)
31 HNF1A (31)
31 AKT2 (31)
28 PIK3R1 (28)
25 MST1R (25)
25 GNAQ (25)
23 TYK2 (23)
22 MAP2K4 (22)
20 RPS6KB1 (20)
19 DDR1 (19)

18 ATRX (18)
16 LCK (16)
15 SLC22A1 (15)
13 SLC22A2 (13)
12 AKT3 (12)
11 IL2RG (11)
11 GNA11 (11)
10 IL2RB (10)
8 DDR2 (8)
7 SUFU (7)
7 PSMB5 (7)
6 SLC31A1 (6)
6 RARB (6)
5 YES1 (5)
5 RXRA (5)
4 ROS1 (4)
3 SLC45A3 (3)
3 SLC34A2 (3)
3 PHF6 (3)
3 ABL2 (3)

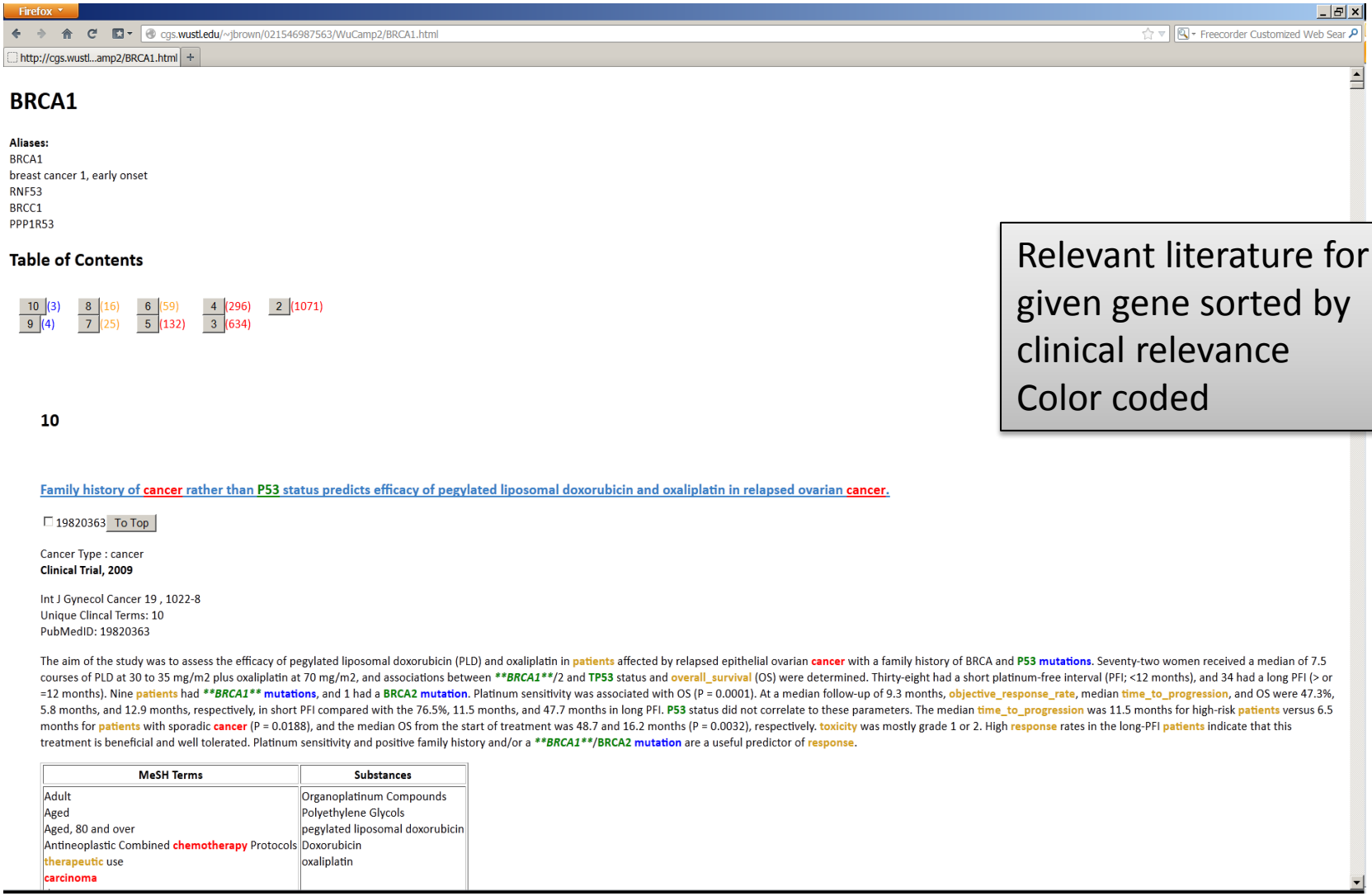
2 RXRB (2)
2 PSMB1 (2)
2 CHIC2 (2)
1 RXRG (1)
1 PSMD2 (1)
1 INPP4B (1)
1 FSTL5 (1)
0 RARG (0)
0 PSMD1 (0
0 PSMB2 (0
0 NELL2 (0)
0 LTK (0)
0 LAMA2 (0
0 H3F3A (0)

Relevant literature broken down by gene

Sorted by annotation worthiness

Human interpretation

Gene Literature



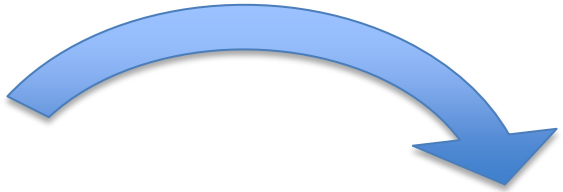
Relevant literature for given gene sorted by clinical relevance
Color coded

http://cgs.wustl.edu/~jbrown/021546987563/WuCamp2/

Search Results Annotation

Inclusion Criteria:

1. Paper must be human clinical data (n >= 7)
2. Paper describe one or more mutations
3. Paper must describe a clinical outcome



Prefiltering	
Total abstracts	10856
Abstracts/hour	8
Total prefilter hours	1357
FTEs	5
Actual prefilter hours	271.4
Actual prefilter weeks	6.8
Actual prefilter months	1.7
Total months	**1.0**

Annotation	
avg accept %	19.26%
tota abs to annot	1426
papers/hr for annot	2
hrs of work	713
days of work	89
FTE	5.00
Total days @5 FTE	17.83
Total weeks @5 FTE	3.57
Total months @5 FTE (Projected)	0.89
Total months actual	**1.0**

Gene	Status	Abstracts	Post-filter	%retained
ERBB2	Clinical, IP issues	1382	126	9
APC	Clinical	1167	86	7
CEBPA	Clinical	1108	26	2
CDKN2A	Clinical, V2 inf	1080	191	18
MYC	Clinical, V2 inf	956	9	1
ATM	Clinical	731	136	19
GSTP1	Clinical	584	166	28
ABCB1	Clinical	552	220	40
VHL	Clinical	497	16	3
ESR1	Clinical	471	47	10
CYP2D6	Clinical	323	52	16
ABL1	Clinical	315	20	6
CYP19A1	Clinical	283	25	9
RB1	Clinical	275	58	21
UGT1A1	Clinical	243	25	10
DPYD	Clinical	220	42	19
NOTCH1	Clinical, Prognostic	147	21	14
CDA	Clinical	130	30	23
TET2	Clinical, Prognostic	85	22	26
ABCC2	Clinical	82	36	44
MPL	Clinical	81	14	17
FGFR4	Clinical, Prognostic	53	27	51
ASXL1	Clinical	46	29	63
MYD88	Clinical	45	2	4
total		10856	1426	19.3%

Variant classification description

- **Level 1** Predictive or prognostic in tumor type (includes inherited cancer susceptibility variants).
- **Level 2** Predictive or prognostic in other tumor type(s).
- **Level 3** Reported in cancer or other disease.
- **Level 4** Variant of unknown significance.
- **Level 5** Known polymorphism.

Variant Classification-1

- Incorporate genomic annotation data to classify variants
  - Chromosome, Gene, mRNA, Protein (NCBI/Ensembl)
  - HGVS nomenclature
    - Consequence (Non-synonymous, Synonymous, etc.)
    - Variant Type/Subtype (Substitution, Indel, etc.)
  - Annotation databases- dbSNP, COSMIC, ClinVar, HGMD, NHLBI ESP, etc.

Variant Classification-1

- Classification strategies and rules
  - Classify SNPs using rules on dbSNP, 1000 Genomes, and NHLBI ESP
  - Classify clinically important variants using clinical tags in dbSNP (OM, CLN, MUT, CDA) and/or using databases such as ClinVar and HGMD
  - Classify disease variants and genes using OMIM or HGMD
  - Classify possible disease causing variants by identifying non-synonymous variants or those that affect splicing
  - Classify possible disease causing variants using in silico predictions (e.g. Condel, SIFT, Polyphen)
  - Classify clinically relevant variants using patient's phenotype(s)

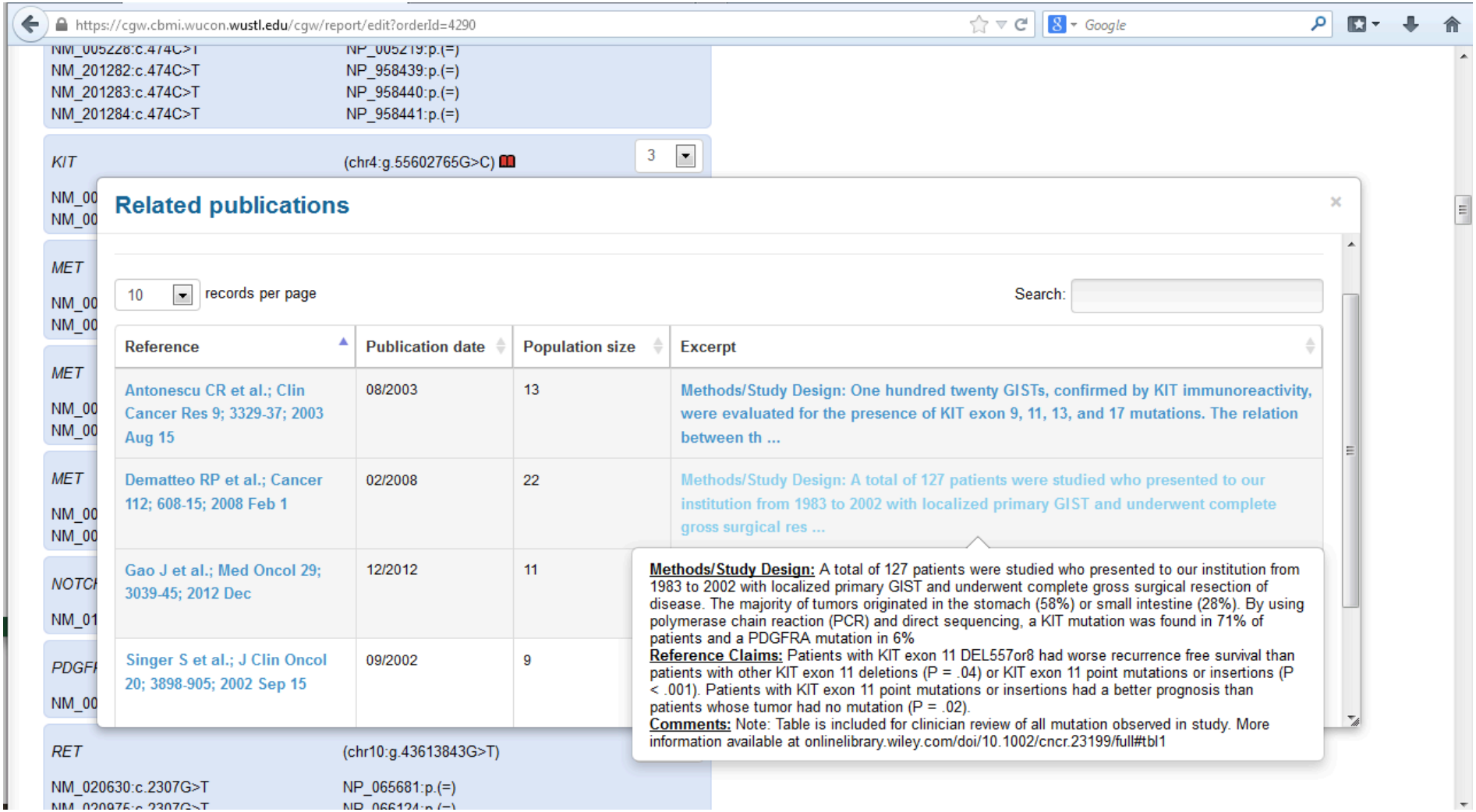
Classification rules (automated)

- If a VariantCall has no other interpretations, but has COSMIC source then classification 3
- If a VariantCall has no other interpretations, but has dbSNP source and (clinical assertions (OM MUT CDA) OR source=ClinVar), then put in class 3
- If a VariantCall has no other interpretations, but has dbSNP source and flags for polymorphism (COMMON=1) but NO (clinical assertions (OM MUT CDA) OR source=ClinVar) then put in class 5
- If a VariantCall has MAF >= 1 for All in NHLBI ESP database, then put in class 5
- If a VariantCall has no other interpretations then set class 4

Variant Classification-2

- Complex decision making using the patient, variant, and variant call fact model
- Variant
  - Gene, mRNA, protein coordinates
  - g, c, p syntax
  - Domains and other functional annotations
  - External variant annotation databases
  - Variant type
- Variant Call
  - Quality criteria
- Patient
  - Disease
  - Drug
  - Phenotype(s)

Variant Classification to interpretation



Example of rules-based variant filtering

- An in frame deletion in exon 11 of *KIT* in GIST responds well to imatinib
  - If deletion AND
  - Consequence is non-synonymous AND
  - p syntax NOT frameshift AND
  - Start and end coordinates in exon 11 of *KIT* AND
  - Patient clinical indication is GIST THEN
  - Infer interpretation

Example of rules-based variant filtering

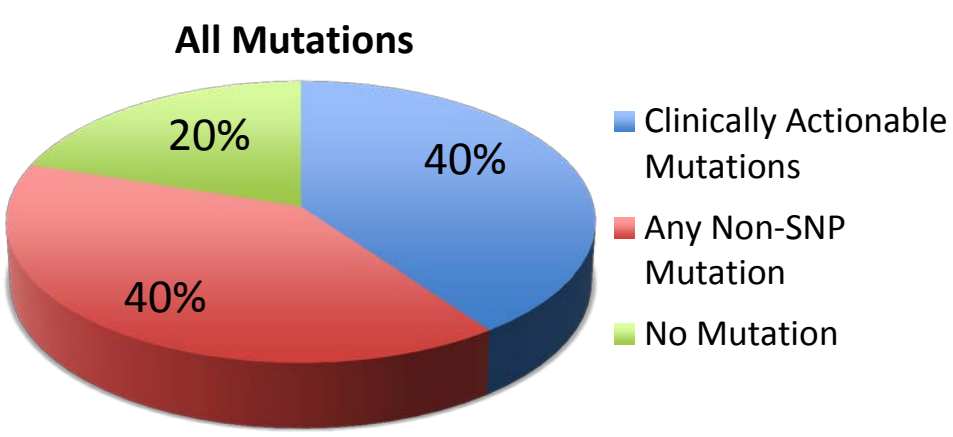
- An in frame deletion in exon 11 of *KIT* in GIST responds well to imatinib EXCEPT if there are secondary mutations in exons 13, 14, 17, or 18
  - If deletion AND
  - Consequence is non-synonymous AND
  - p syntax NOT frameshift AND
  - Start and end coordinates in exon 11 of *KIT* AND
  - NOT Any non-synonymous variant in *KIT* in exons 13, 14, 17, or 18
    - FOR each variant called in this specimen:
      - Consequence is non-synonymous AND
      - Start and end coordinates in exon 13 OR 14 OR 17 OR 18 of *KIT*
  - Patient clinical indication is GIST THEN
  - Infer interpretation

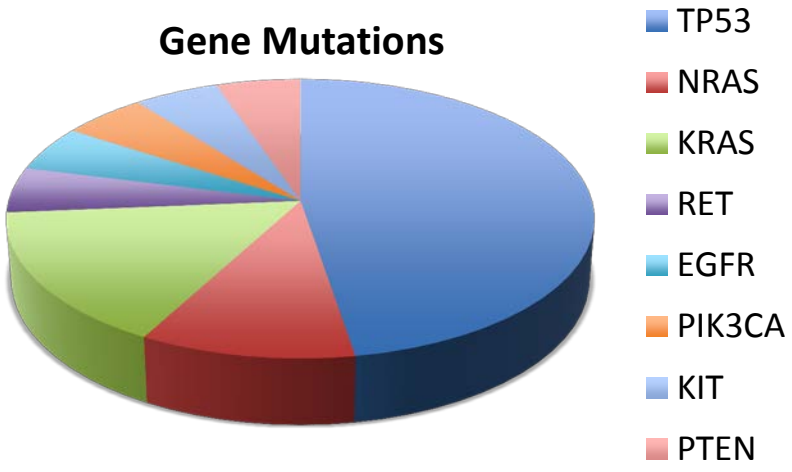
Actionability

1. Practice guidelines for the genetic condition exist
2. Professional society practice guidelines recommends action in
   - patient management
   - surveillance or screening
   - family management
   - circumstances to avoid
3. Medical interventions based on new results are effective
4. Actions are acceptable to individual in terms of burdens or risks

Cancer Panel Initial Findings

- ~1500 clinical tests ordered since March, 2012 (does not include clinical trials)
- ~45% of sequenced cases have specific 'actionable' mutations in targetable genes including *KRAS*, *EGFR*, *KIT*, and *PIK3CA*.





Presentation of the results

Washington University Physicians
Washington University School of Medicine in St. Louis

Clinical Genomics
Department of Pathology and Immunology
Phone: 314-454-8101; Fax: 314-454-5192
http://cytogenomics.wustl.edu http://clinicalgenomics.wustl.edu
CLIA #26D0698685; CAP# 27556-03

Name:
Gender:
DOB:
Tissue:

MRN:
Hospital #:
Facility:

Culture #:
Date Collected:
Date Accessioned:
Date Ordered:

Physician(s):

Processing:
Indication:
Specimen Quality:

GENOMIC SEQUENCING

TREATMENT REFRACTORY SET

Summary

A 15bp, in-frame exon 19 deletion was detected in *EGFR*, and is predicted to be sensitive to EGFR-inhibitors (Mitsudomi T; J Clin Oncol; 2513-20; 2005 Apr 10) (Kosaka T; Cancer Res; 8919-23; 2004 Dec 15). This deletion was confirmed by Sanger sequencing, and has been previously reported by indpendent testing (see S11-51638).

A nonsense mutation in exon 1 of the tumor suppressor *PTEN* (p.Q17*) was identified, which has been reported in other tumor types; this mutation is of unknown clinical significance. The allele frequency of this mutation suggests that it may be homozygous in the tumor tissue.

The identified *TP53* (p.R116Q) single base substitution has been previously reported in non-small cell lung carcinoma, and is of unknown clinical significance.

Unique sequence variants of unknown significance are reported below. The sequence analysis and interpretations were reviewed by Drs. C , who concur.

Pathologist review (performed by) of an H&E stained section of the tissue block was used to guide microdissection of areas of viable tumor by the Washington University AMP Core Labs. DNA was extracted from the areas of viable tumor for sequence analysis. The technical component of the testing passed all established laboratory QC metrics except exon 2 of *DNMT3A,* exon 1 of *MAP2K2,* exon 1 of *MAPK1,* exon 1 of *KRAS,* exon 1 of *RET,* exon 1 of *WT1,* where variants may not have been reliably detected.

Challenges and approaches

Challenges and Opportunities

- Interpretation of genomic data is difficult and labor intensive
  - A blend of algorithmic and knowledge-based variant curation methods necessary for clinical interpretation
  - Annotating and keeping up with variant management is expensive
  - Variants need to be linked with **clinical outcomes**
- Urgent need for implementation of universal standards and variant resource databases
  - Clinical Genome Resource (ClinGen)
  - High level inventory of key aberrations in cancer with clinical correlation does not exist

GENOMICS AND PATHOLOGY SERVICES

GPS@WUSTL

CLINICAL TESTING • RESEARCH • EDUCATION

Karen Seibert, John Pfiefer, Skip Virgin,

Jeffrey Millbrandt, Rob Mitra, Rich Head

Rakesh Nagarajan and his Bioinf. team

David Spencer, Eric Duncavage, Andy Bredm.

Hussam Al-Kateb, Cathy Cottrell

Dorie Sher, Jennifer Stratman

Tina Lockwood, Jackie Payton

Mark Watson, Seth Crosby, Don Conrad

Andy Drury, Kris Rickhoff

Mike Isaacs and his IT Team

Norma Brown, Cherie Moore,

Magie O Guin, GTAC team

Jane Bauer and Cytogenomics &Mol path team

MANY MORE!