

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



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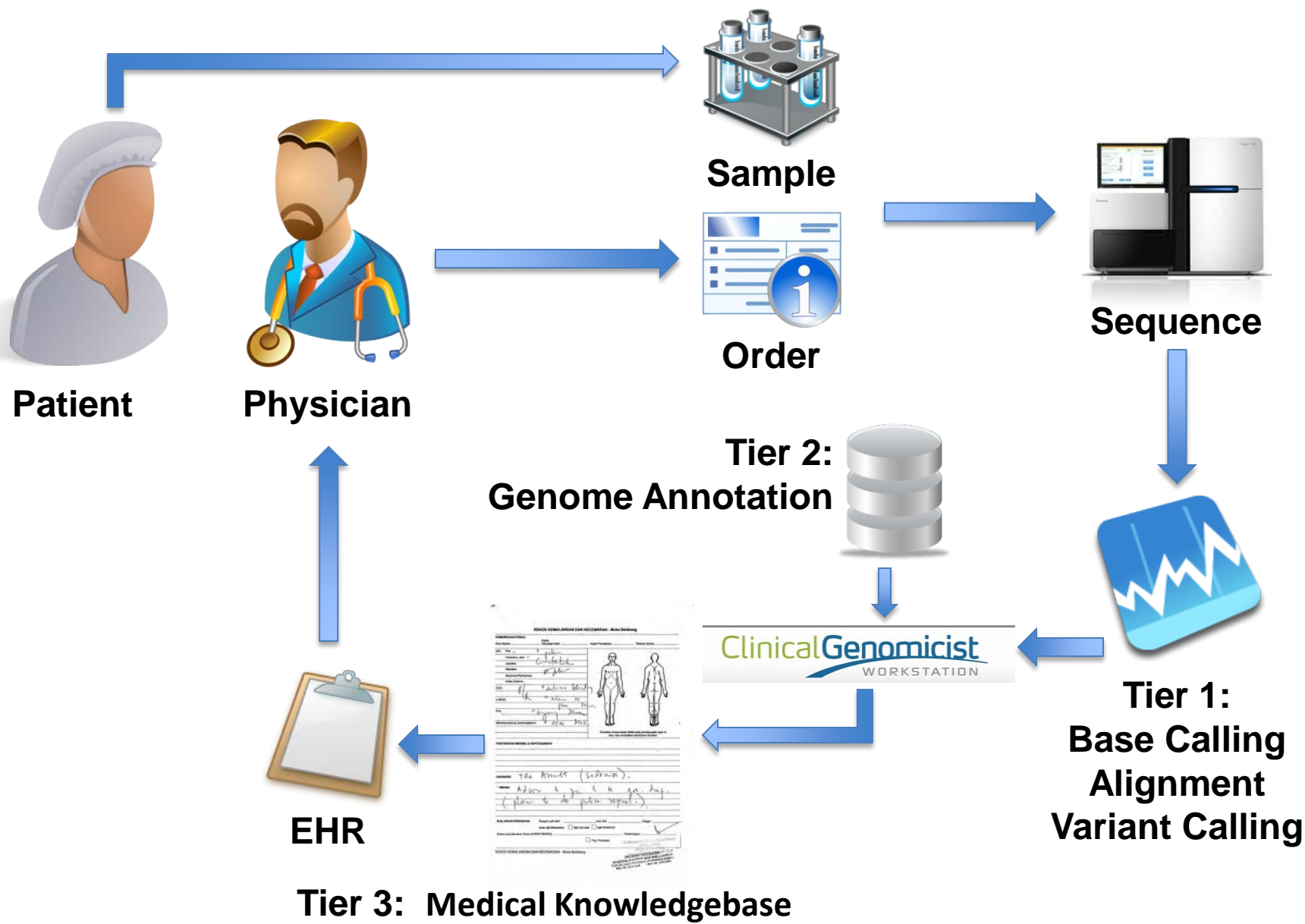
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A National Cancer Institute Comprehensive Cancer Center

Evidence-based review of variants

Introduction to Workflow and
process

NGS testing workflow (Clinical Genomicist workstation- CGW)

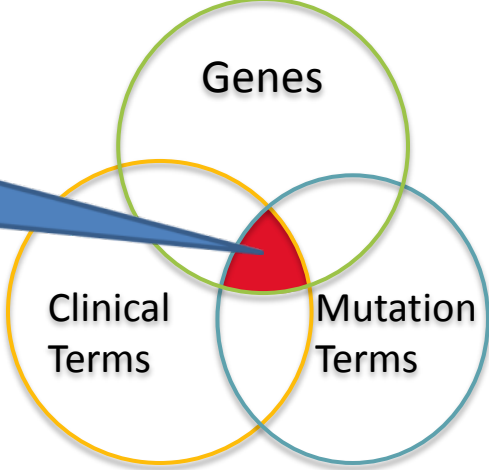


Critical assessment of variants

Variant analysis and filtration

Automated variant searches and selection process

Automated Searches



n=8261

Prefiltering

Inclusion Criteria n=1519 (25%)

Annotation Worthy n << 1519

Annotation

Interpretation

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	2131410
ABCB1.3; ABCB1.4; ABCB1.5; ABCB1.7	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; ABCB1.7	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; ABCB1.7	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	To investigate the relationship of ABCB1	clinical data			The ABCB1 T236CT/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 significant			
ABCB1.3; ABCB1.4; ABCB1.5; ABCB1.7	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; ABCB1.7	T1236>C; T2677>G; T2677>A;	G412G; S893A; S893T; I1145I	Cancer	Imatinib	NOT SIGNIFICANT: We investigated the	clinical data			ABCB1 polymorphism was not significant			

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)	603 NRAS (603)	292 AKT1 (292)	114 STK11 (114)	49 SMO (49)	18 ATRX (18)	2 RXRB (2)
3087 KRAS (3087)	584 GSTP1 (584)	283 CYP19A1 (283)	104 FGFR2 (104)	46 JAK1 (46)	16 LCK (16)	2 PSMB1 (2)
2626 TP53 (2626)	552 ABCB1 (552)	273 RB1 (273)	102 CYP2C19 (102)	46 ASXL1 (46)	15 SLC22A1 (15)	2 CHIC2 (2)
2240 BRCA1 (2240)	531 MTOR (531)	243 UGT1A1 (243)	99 PTPN11 (99)	45 MYD88 (45)	13 SLC22A2 (13)	1 RXRG (1)
1717 BRCA2 (1717)	503 TAS2R38 (503)	243 HRAS (243)	98 ERG (98)	39 JAK3 (39)	12 AKT3 (12)	1 PSMD2 (1)
1591 KIT (1591)	497 VHL (497)	233 IDH1 (233)	86 SMARCB1 (86)	38 MAP2K2 (38)	11 IL2RG (11)	1 INPP4B (1)
1481 BRAF (1481)	482 JAK2 (482)	220 DPYD (220)	85 TET2 (85)	38 ERBB4 (38)	11 GNA11 (11)	1 FSTL5 (1)
1382 ERBB2 (1382)	471 ESR1 (471)	206 IL2RA (206)	84 SHH (84)	36 SLCO1B1 (36)	10 IL2RB (10)	0 RARG (0)
1185 RET (1185)	393 RUNX1 (393)	205 CDKN2B (205)	82 CYP2C9 (82)	35 MAP3K1 (35)	8 DDR2 (8)	0 PSMD1 (0)
1166 APC (1166)	374 NPM1 (374)	204 CEBPA (204)	82 ABCC2 (82)	33 FLT1 (33)	7 SUFU (7)	0 PSMB2 (0)
1084 PTEN (1084)	354 SRC (354)	186 SMAD4 (186)	81 MPL (81)	33 CYP2B6 (33)	7 PSMB5 (7)	0 NELL2 (0)
1080 CDKN2A (1080)	352 PDGFRA (352)	177 KDR (177)	78 ERBB3 (78)	31 HNF1A (31)	6 SLC31A1 (6)	0 LTK (0)
1043 MLH1 (1043)	351 WT1 (351)	176 PDGFRB (176)	77 CSF1R (77)	31 AKT2 (31)	6 RARB (6)	0 LAMA2 (0)
956 MYC (956)	323 CYP2D6 (323)	154 CDH1 (154)	76 RARA (76)	28 PIK3R1 (28)	5 YES1 (5)	0 H3F3A (0)
942 PIK3CA (942)	315 ABL1 (315)	147 NOTCH1 (147)	70 CYP2A6 (70)	25 MST1R (25)	5 RXRA (5)	
899 FLT3 (899)	308 NF1 (308)	143 FGFR3 (143)	59 GNAS (59)	25 GNAQ (25)	4 ROS1 (4)	
881 MET (881)	304 FBXW7 (304)	131 PTCH1 (131)	53 FGFR4 (53)	23 TYK2 (23)	3 SLC45A3 (3)	
731 ATM (731)	300 MLL (300)	130 CDA (130)	53 DNMT3A (53)	22 MAP2K4 (22)	3 SLC34A2 (3)	
717 CTNNB1 (717)	298 FGFR1 (298)	122 ESR2 (122)	52 FLT4 (52)	20 RPS6KB1 (20)	3 PHF6 (3)	
655 MAPK1 (655)	294 ALK (294)	120 IDH2 (120)	51 EZH2 (51)	19 DDR1 (19)	3 ABL2 (3)	

Relevant literature
broken down by gene

Sorted by annotation
worthiness

Human interpretation

Gene Literature

Firefox

cgswustl.edu/~jbrown/021546987563/WuCamp2/BRCA1.html

http://cgswustl...amp2/BRCA1.html

BRCA1

Aliases:
BRCA1
breast cancer 1, early onset
RNF53
BRCC1
PPP1R53

Table of Contents

10 (3)	8 (16)	6 (59)	4 (296)	2 (1071)
9 (4)	7 (25)	5 (132)	3 (634)	

10

[Family history of cancer rather than P53 status predicts efficacy of pegylated liposomal doxorubicin and oxaliplatin in relapsed ovarian cancer.](#)

19820363 [To Top](#)

Cancer Type : cancer
Clinical Trial, 2009

Int J Gynecol Cancer 19 , 1022-8
Unique Clinical Terms: 10
PubMedID: 19820363

The aim of the study was to assess the efficacy of pegylated liposomal doxorubicin (PLD) and oxaliplatin in patients affected by relapsed epithelial ovarian cancer with a family history of BRCA and P53 mutations. Seventy-two women received a median of 7.5 courses of PLD at 30 to 35 mg/m² plus oxaliplatin at 70 mg/m², and associations between **BRCA1**/2** and **TP53** status and **overall_survival** (OS) were determined. Thirty-eight had a short platinum-free interval (PFI; <12 months), and 34 had a long PFI (>=12 months). Nine patients had **BRCA1** mutations**, and 1 had a **BRCA2 mutation**. Platinum sensitivity was associated with OS (P = 0.0001). At a median follow-up of 9.3 months, **objective_response_rate**, median **time_to_progression**, and OS were 47.3%, 5.8 months, and 12.9 months, respectively, in short PFI compared with the 76.5%, 11.5 months, and 47.7 months in long PFI. **P53** status did not correlate to these parameters. The median **time_to_progression** was 11.5 months for high-risk patients versus 6.5 months for patients with sporadic cancer (P = 0.0188), and the median OS from the start of treatment was 48.7 and 16.2 months (P = 0.0032), respectively. **toxicity** was mostly grade 1 or 2. High **response** rates in the long-PFI patients indicate that this treatment is beneficial and well tolerated. Platinum sensitivity and positive family history and/or a **BRCA1**/BRCA2 mutation** are a useful predictor of **response**.

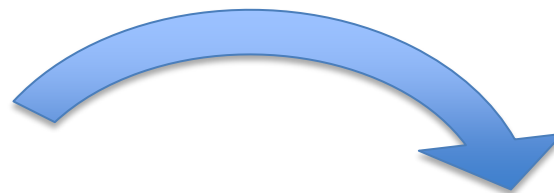
MeSH Terms	Substances
Adult	Organoplatinum Compounds
Aged	Polyethylene Glycols
Aged, 80 and over	pegylated liposomal doxorubicin
Antineoplastic Combined chemotherapy Protocols	Doxorubicin
therapeutic use	oxaliplatin
carcinoma	

Relevant literature for given gene sorted by clinical relevance
Color coded

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

Browser address bar: <https://cgw.cbmi.wucon.wustl.edu/cgw/report/edit?orderId=4290>

Variant list:

- NM_005228:c.474C>T NP_005219:p.(=)
- NM_201282:c.474C>T NP_958439:p.(=)
- NM_201283:c.474C>T NP_958440:p.(=)
- NM_201284:c.474C>T NP_958441:p.(=)

Gene: **KIT** (chr4:g.55602765G>C) 3

Related publications

10 records per page Search:

Reference	Publication date	Population size	Excerpt
Antonescu CR et al.; Clin Cancer Res 9; 3329-37; 2003 Aug 15	08/2003	13	Methods/Study Design: One hundred twenty GISTs, confirmed by KIT immunoreactivity, were evaluated for the presence of KIT exon 9, 11, 13, and 17 mutations. The relation between th ...
Dematteo RP et al.; Cancer 112; 608-15; 2008 Feb 1	02/2008	22	Methods/Study Design: A total of 127 patients were studied who presented to our institution from 1983 to 2002 with localized primary GIST and underwent complete gross surgical res ...
Gao J et al.; Med Oncol 29; 3039-45; 2012 Dec	12/2012	11	Methods/Study Design: A total of 127 patients were studied who presented to our institution from 1983 to 2002 with localized primary GIST and underwent complete gross surgical resection of disease. The majority of tumors originated in the stomach (58%) or small intestine (28%). By using polymerase chain reaction (PCR) and direct sequencing, a KIT mutation was found in 71% of patients and a PDGFRA mutation in 6% Reference Claims: Patients with KIT exon 11 DEL557or8 had worse recurrence free survival than patients with other KIT exon 11 deletions (P = .04) or KIT exon 11 point mutations or insertions (P < .001). Patients with KIT exon 11 point mutations or insertions had a better prognosis than patients whose tumor had no mutation (P = .02). Comments: Note: Table is included for clinician review of all mutation observed in study. More information available at onlinelibrary.wiley.com/doi/10.1002/cncr.23199/full#tbl1
Singer S et al.; J Clin Oncol 20; 3898-905; 2002 Sep 15	09/2002	9	

Gene: **RET** (chr10:g.43613843G>T)

Variant list:

- NM_020630:c.2307G>T NP_065681:p.(=)
- NM_020975:c.2307G>T NP_066124:p.(=)

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

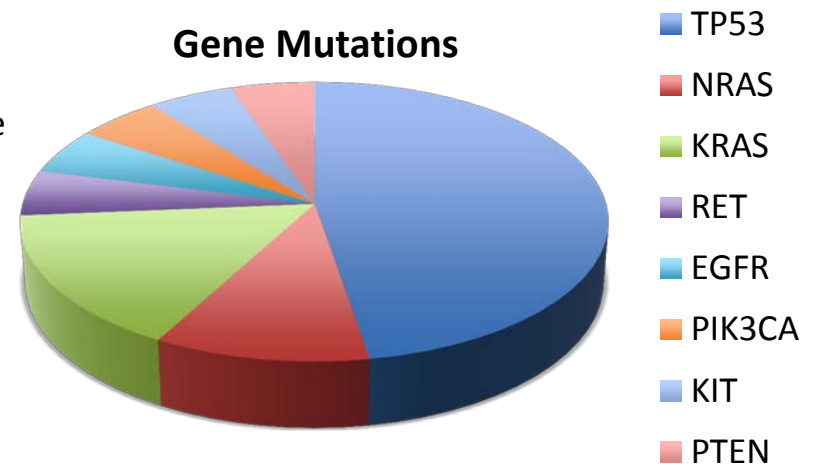
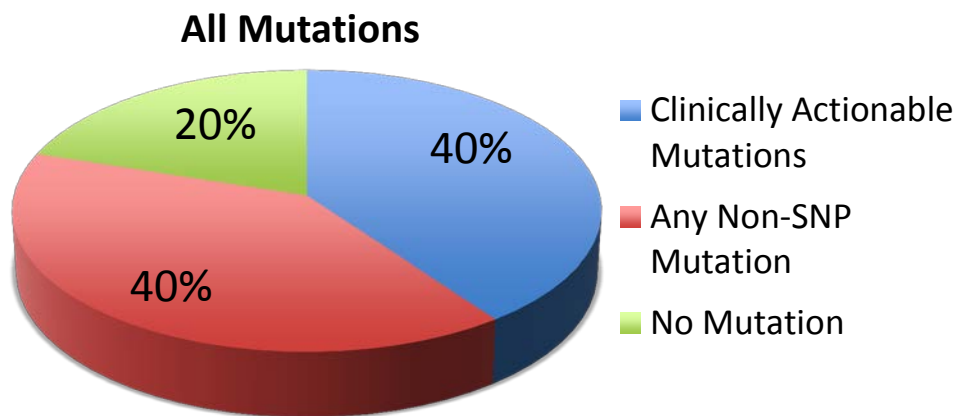
- An in frame deletion in exon 11 of *KIT* in GIST responds well to imatinib EXCEPT if there are secondary mutations in exons 13, 14, 17, or 18
 - If deletion AND
 - Consequence is non-synonymous AND
 - p syntax NOT frameshift AND
 - Start and end coordinates in exon 11 of *KIT* AND
 - NOT Any non-synonymous 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



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:	MRN:	Culture #:
Gender:	Hospital #:	Date Collected:
DOB:	Facility:	Date Accessed:
Tissue:		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 independent 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.

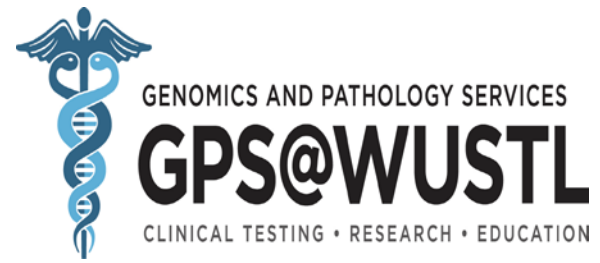
Unique sequence variants of unknown significance are reported below. The sequence analysis and interpretations were reviewed by Drs. C _____, who concur.

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



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!

