The Cancer Genomics Resource List 2014

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• ***Context.***—**Genomic sequencing for cancer is offered by commercial for-profit laboratories, independent laboratory networks, and laboratories in academic medical centers and integrated health networks. The variability among the tests has created a complex, confusing environment.**

Objective.—**To address the complexity, the Personalized Health Care (PHC) Committee of the College of American Pathologists proposed the development of a cancer genomics resource list (CGRL). The goal of this resource was to assist the laboratory pathology and clinical oncology communities.**

Design.—**The PHC Committee established a working group in 2012 to address this goal. The group consisted of site-specific experts in cancer genetic sequencing. The group identified current next-generation sequencing (NGS)–based cancer tests and compiled them into a usable resource. The genes were annotated by the working group. The annotation process drew on published knowledge, including public databases and the medical literature.**

Results.—**The compiled list includes NGS panels offered by 19 laboratories or vendors, accompanied by annotations. The list has 611 different genes for which NGS-based mutation testing is offered. Surprisingly, of these 611 genes, 0 genes were listed in every panel, 43 genes were listed in 4 panels, and 54 genes were listed in 3 panels. In addition, tests for 393 genes were offered by only 1 or 2 institutions. Table 1 provides an example of gene mutations offered for breast cancer genomic testing with the annotation as it appears in the CGRL 2014.**

Conclusions.—**The final product, referred to as the Cancer Genomics Resource List 2014, is available as supplemental digital content.**

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The last 10 years have seen a transformation in genomic medicine and its application to cancer research and clinical care.[1,2] Comprehensive sequencing efforts have identified at least 140 genes that are somatically mutated in cancer,[3] some of which can promote or drive tumorigenesis. In several cases, knowledge of driver mutations has led to the development of specific inhibitors, which has profoundly altered patient care.[4,5] In addition to tumor-specific mutations that define the response to a targeted therapy, the same or other mutations in the tumor may be associated with diagnosis[6] and/or prognosis.[7,8] Constitutional variants may also have predictive, prognostic, or, in some cases, diagnostic value (eg, germline cancer predisposition variants).

Increased discoveries in cancer genomics have been facilitated by a precipitous decline in the cost of sequencing. Whole-genome sequencing, formerly a billion-dollar proposition, can now be accomplished for $10 000 or less, and the "$1000 exome" is becoming a reality. The time required for sequencing has also decreased. Genomic data can now be obtained in days rather than years, making this information available in a clinically relevant time frame. Therefore, interest in and demand for the integration of genomic medicine into routine care has accelerated exponentially, driven by diverse stakeholders including pathologists, oncologists, and their patients.[9]

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Table 1. Example of Gene Mutations Offered for Breast Cancer Genomic Testing[a,b]

Gene	Tumor Type	Mutation Type	Expected Frequency
ABL1	Carcinoma	Mutation, amplification (e7-1, Y459H, D501E, V741fs)	
ABL2	Carcinoma	Mutation, amplification (D227Y, R612K)	
AKT1	Carcinoma	Mutation, amplification (E17K [11], L52R)	2%–6%
AKT2	Carcinoma	Mutation, amplification (E356K)	
AKT3	Carcinoma	Mutation, amplification (R66X, P310A, S375X)	
ALK	Carcinoma	Mutation, amplification (G843R, R1275X, FGM1271in_frame_delL)	
AR	Carcinoma	Polymorphisms/germline mutation	Rare
ATM	Carcinoma	Mutation	Constitutional, no basal type
AURKA	Carcinoma	Polymorphism/constitutional single-nucleotide polymorphism F31I	
BRAF	Carcinoma	Mutation, amplification (K698R, L537S, E309X)	
BRCA1	Carcinoma	Mutation (many nonsense, frameshift, or missense mutations); deletions	Constitutional, no *HER2/neu* enriched, mostly basal
BRCA2	Carcinoma	Mutation (many nonsense, frameshift, or missense mutations); deletions	Constitutional, no *HER2/neu* enriched
BRIP1	Carcinoma	Mutation	Constitutional, rare
CBFB		Mutation	2%–4%
CCND1	Carcinoma	Amplification (R260C mutation)	
CCND3	Carcinoma	Mutation	
CDH1	Carcinoma	Mutation	6.5% overall, 10% luminal A
CDK12	Carcinoma: ER-positive	Silencing/inactivation of gene	
CDK4	Carcinoma	Amplification	
CDK6	Carcinoma	Amplification; mutation (H139Q)	
CDKN1B	Carcinoma	Mutation	
CHEK2	Carcinoma	Mutation	Constitutional
CTCF	Carcinoma	Mutation	
DDR2	Carcinoma	Mutation, amplification (S123I, E361D, A407P, K616N, S674Y, R752H)	
EPHA5	Triple negative	Translocation	Rare
ERBB2	Carcinoma	Activating mutations, amplification/overexpression, decreased phosphorylation (by PTPN12)	
ERBB3	Carcinoma	Mutations, amplification (F94L, Q153X, D297Y, T355I, A378P, L783V, L792V, T1169P, E1261A)	
ERBB4	Carcinoma	Mutations, amplification (D73V, P172R, G500E, T703K, E1205K, P1292S)	
ESR1	Carcinoma	Activating mutation in ligand-binding domain	
ETV5	Carcinoma	Multiple	
EZH2	Carcinoma	Increased expression	
FANCC	Carcinoma	Single-nucleotide polymorphism	Rare
FGFR2	Carcinoma	rs11200014, rs2981579, rs1219648, rs2420946 (intron 2); amplification in basal subtype	
FGFR3	Carcinoma	Mutation, amplification (P688S)	
FGFR4	Carcinoma	Mutation, amplification (E326K, A484T, E741fs)	
GATA3	Carcinoma	Mutation	4%–11% (47% luminal A, 32% luminal B)
GSTM5	Carcinoma	Many nonsynonymous single-nucleotide polymorphisms	
INHBA	Multiple	Promoter methylation changes, overexpression	
JAK1	Carcinoma	Mutation, amplification (G600W, K1090Q)	
JAK2	Carcinoma	Mutation, amplification (Y44X, L55P, R115I, Y132X, N646fs)	
JAK3	Carcinoma	Mutation, amplification (F110fs, R741Q)	
KIT	Carcinoma	Mutation, amplification (P36T, I39M, A219V, A280V, G432E)	

Table 1. Extended

Gene	No. of Panels in Which Gene Is Listed[c]	Helpful Refs	Therapeutic, Diagnostic, Prognostic Target (Yes, No, Unknown)	Notes
ABL1	18		Unk, Unk, Unk	
ABL2	2		Unk, Unk, Unk	
AKT1	23	Banerji et al,[151] 2012; Cancer Genome Atlas Network,[18] 2012	Yes, No, No	
AKT2	6		Unk, Unk, Unk	
AKT3	7		Unk, Unk, Unk	
ALK	23		Unk, Unk, Unk	
AR	4	Dimitrakakis and Bondy,[152] 2009	Yes, No, Yes	
ATM	27		Unk, Unk, Unk	
AURKA	4		Unk, No, No	Therapeutic–hereditary breast cancer risk
BRAF	25	Chapman et al,[104] 2011; Sosman et al,[153] 2012; Hauschild et al,[154] 2012	Unk, Unk, Unk	
BRCA1	13		Unk, Unk, Unk	
BRCA2	13		Unk, Unk, Unk	
BRIP1	9		Unk, Unk, Unk	
CBFB	1	Banerji et al,[151] 2012; Cancer Genome Atlas Network,[18] 2012	No, No, No	
CCND1	4		Unk, Unk, Unk	
CCND3	3		Unk, Unk, Unk	
CDH1	27		Unk, Unk, Unk	
CDK12	2	Iorns et al,[150] 2009	Yes, No, Yes	Decreased CDK12 is associated with resistance to tamoxifen, and possibly with increased efficacy of PARP1/2 inhibitors
CDK4	8		Unk, Unk, Unk	
CDK6	4		Unk, Unk, Unk	
CDKN1B	2		Unk, Unk, Unk	
CHEK2	10		Unk, Unk, Unk	
CTCF	1		Unk, Unk, Unk	
DDR2	8		Unk, Unk, Unk	
EPHA5	3		Unk, Unk, Unk	
ERBB2	20		Yes, Unk, Unk	In situ hybridization is the clinically validated technology for assessing *ERBB2* amplification. NGS-based methods must also probe the centromere of chromosome 17 to distinguish between polysomy 17 and amplification of *ERBB2*; therapeutic–Y breast, gastric
ERBB3	4		Unk, Unk, Unk	
ERBB4	15		Unk, Unk, Unk	
ESR1	5	Toy et al,[155] 2013	Yes, No, No	ER antagonists such as tamoxifen
ETV5	2	Oh et al,[48] 2012	Unk, Unk, Unk	May be useful in prognosis
EZH2	15		Unk, No, No	
FANCC	5	Thompson et al,[156] 2012	No, No, No	Constitutional susceptibility in *BRCA*-negative patients
FGFR2	19	Hunter et al,[160] 2007	Unk, Unk, Unk	Predictive target
FGFR3	17		Unk, Unk, Unk	
FGFR4	7		Unk, Unk, Unk	
GATA3	3	Banerji et al,[151] 2012; Cancer Genome Atlas Network,[18] 2012	No, No, No	
GSTM5	1		Unk, Unk, Unk	Part of polymorphic gastrointestinal stromal tumor family, polymorphisms associated with breast/prostate cancer
INHBA	1		Unk, Unk, Unk	Involved in proliferation, overexpression associated with poor prognosis
JAK1	5		Unk, Unk, Unk	
JAK2	18		Unk, Unk, Unk	
JAK3	16		Unk, Unk, Unk	
KIT	23		Unk, Unk, Unk	

Table 1. Extended

Gene	A	B	C	D	E1	E2	E3	E4	F	G	H1	H2	H3	H4	I1	I2	J1	J2	J3	J4	J5	J6	J7	J8
ABL1	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	N	Y	Y	N	N	N	N	N	N	N	N
ABL2	N	N	N	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
AKT1	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	N	N	N	N	N
AKT2	N	Y	Y	N	Y	N	N	N	N	Y	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N
AKT3	N	Y	Y	Y	Y	N	N	N	N	Y	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N
ALK	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	N	Y	Y	N	N	N	N	N	N	N	N
AR	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N
ATM	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	Y	Y	Y	Y	N	Y	N	Y	N	Y	N
AURKA	N	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BRAF	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	N	N	N	N	N
BRCA1	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	N	Y	N	Y	N
BRCA2	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	N	Y	N	Y	N
BRIP1	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	N	Y	N	N	N
CBFB	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CCND1	N	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CCND3	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CDH1	N	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y	Y	N	N	N
CDK12	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CDK4	N	Y	Y	N	Y	N	N	N	N	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N
CDK6	N	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CDKN1B	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CHEK2	N	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	Y	Y	N	N	N
CTCF	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
DDR2	N	Y	Y	N	Y	N	Y	N	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N
EPHA5	N	Y	Y	N	N	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ERBB2	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	N	N	Y	N	N	N	N	N	N	N	N
ERBB3	N	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ERBB4	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	N	N	Y	N	N	N	N	N	N	N	N
ESR1	Y	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ETV5	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
EZH2	N	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
FANCC	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FGFR2	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	N	Y	Y	N	N	N	N	N	N	N	N
FGFR3	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
FGFR4	Y	Y	Y	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GATA3	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GSTM5	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
INHBA	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
JAK1	N	Y	N	N	Y	N	N	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
JAK2	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	N	Y	Y	N	N	N	N	N	N	N	N
JAK3	N	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N
KIT	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	N	N	N	N	N	N	N	N

Table 1. Extended

Gene	K	L1	L2	L3	M1	M2	N1	N2	N3	N4	N5	N6	O	P1	P2	Q	R1	R2	R3	S1	S2
ABL1	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	N	Y	Y
ABL2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
AKT1	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	Y	N	Y	Y	Y	Y
AKT2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
AKT3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALK	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	Y	Y	Y	Y	Y
AR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ATM	Y	Y	N	Y	N	Y	N	N	N	Y	Y	Y	Y	N	Y	N	Y	Y	N	N	Y
AURKA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
BRAF	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	Y	N	Y	Y	Y	Y
BRCA1	N	N	N	N	N	N	Y	N	N	Y	Y	Y	N	N	N	N	Y	N	N	N	N
BRCA2	N	N	N	N	N	N	Y	N	N	Y	Y	Y	N	N	N	N	Y	N	N	N	N
BRIP1	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N
CBFB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CCND1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CCND3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
CDH1	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y	N	N
CDK12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CDK4	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	Y	N	N	N	N
CDK6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CDKN1B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CHEK2	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
CTCF	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
DDR2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N
EPHA5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ERBB2	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	Y	Y	N
ERBB3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ERBB4	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	N	N	N
ESR1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ETV5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
EZH2	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	Y	N	N	N	Y
FANCC	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	Y	N	N	N	N
FGFR2	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	Y	Y	Y
FGFR3	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	N	Y	Y
FGFR4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y
GATA3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
GSTM5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
INHBA	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
JAK1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
JAK2	N	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	N	N	N	Y	N	N	Y
JAK3	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	N	N	Y
KIT	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	Y	Y	Y	Y	Y

Table 1. Continued

Gene	Tumor Type	Mutation Type	Expected Frequency
LTK	Carcinoma	Mutation, amplification (E374D)	
MAP2K4	Carcinoma	Mutation	4% overall; 7% luminal A
MAP3K1	Carcinoma: luminal A–type breast cancer	R364G, H393fs, H393Q, S431fs, Q957stop, R1012fs, L1052fs, M1269fs, E1286in_frame_del, S1344stop, V1346in_frame_del, Q1494stop	3%–8%, (14%–15% of luminal tumors)
MET	Carcinoma	Mutation, amplification (R412G, G225D, (RS359in_frame_delP+ A361in_frame_del+ M362fs)	Low
MLL3	Carcinoma	Mutation	≈7%
MYB	Carcinoma	Mutation	Rare luminal A
NBN	Carcinoma	Mutation	Constitutional
NF1	Carcinoma	Mutation	
NOTCH1	Carcinoma	Polymorphisms, translocation	60% T-ALL
PDGFRA	Carcinoma	Mutation, amplification (E571K, R822H, R981P)	
PDGFRB	Carcinoma	Mutation, amplification (D453E, R437H)	
PIK3CA			27%–36% (45% in luminal A)
PIK3CB	ER-positive tumors	Amplification	0.05
PIK3R1	Carcinoma	Most common mutations in region of 456–469 and 564–575	
PTEN	Carcinoma	Mutation (e5-1, e6+2, e6-2, E288fs, T319fs, HY64in_frame_del, K128N, K266X); deletions	Constitutional, 3% overall
PTPRD	Carcinoma	Mutation	
RAD50	Carcinoma	Point mutations, small indels, large deletions	Low
RAD51C	Carcinoma	Mutation	Constitutional, basal
RB1	Carcinoma	Mutation	
ROS1	Carcinoma	Mutation, amplification (S551F, E596K, e41+2, N2234K, T2266R)	
RUNX1	Carcinoma	Mutation	0.035
SF3B1	Carcinoma	Mutation	
SOCS1	Carcinoma	Altered gene expression (higher in some cancers, while lower in others), not well understood	
SYK	T-cell lymphoma-positive carcinoma	Translocation for T-cell lymphoma; heritable single-nucleotide polymorphism for increased risk in breast cancer	7-fold increased risk in some breast cancer populations
SYK	Carcinoma	Translocation for T-cell lymphoma; heritable single-nucleotide polymorphisms for increased risk in breast cancer	7-fold increased risk in some breast cancer populations
TLR4	Carcinoma	Mutation	Rare, ER-positive, *HER2/neu*-negative
TNFAIP3	Carcinoma	Overexpression	
TP53	Carcinoma	Mutation	Constitutional, 27%–37% (80% basal, 74% *HER2/neu* enriched)

Abbreviations: ER, estrogen receptor; GIST, gastrointestinal stromal tumor; *HER2/neu*, gene for human epidermal growth factor receptor; N, no; NGS, next-generation sequencing; PARP, poly-(ADP-ribose)-polymerase; Refs, references; T-ALL, T-cell acute lymphocytic leukemia; Unk, unknown; Y, yes; A, Washington University (St Louis, Missouri) – Comprehensive Cancer Gene Set, v2; B, Foundation Medicine – FoundationOne (Cambridge, Massachusetts); C, Brigham & Women's Hospital (Boston, Massachusetts); D, Qiagen GeneRead DNAseq Human Comprehensive Cancer Panel (Germantown, Maryland); E, Life Technologies Ion AmpliSeq (Carlsbad, California), E1: Comprehensive Cancer Panel, E2: Cancer Hotspot Panel v2, E3: Colon and Lung Cancer Panel, E4: AML Panel; F, Baylor College of Medicine (Houston, Texas); G, University of Washington (Seattle, Washington) – Oncoplex Panel; H, Knight Diagnostic Laboratories (Portland, Oregon), H1: GeneTrails NSCLC Genotyping Panel, H2: GeneTrails Solid Tumor Panel, H3: GeneTrails AML MDS Genotyping Panel, H4: GeneTrails GIST Genotyping Panel; I, Caris Life Sciences (Irving, Texas), I1: Molecular Intelligence for Solid Tumors, I2: Molecular Intelligence by Next-Generation Sequencing; J, Ambry Genetics (Aliso Viejo, California), J1: CancerNext, J2: BRCAPlus, J3: BreastNext, J4: ColoNext, J5: OvaNext, J6: RenalNext, J7: PancNext, J8: PGLNext; K, ARUP Laboratories (Salt Lake City, Utah) – Solid Tumor; L, Montefiore Medical Center (Bronx, New York), L1: Hot Spot, L2: Other somatic genes, L3: Other germline genes; M: Mayo Medical Laboratories (Rochester, Minnesota), M1: Colorectal Cancer Panel, M2: 50-Gene Panel; N, Invitae (San Francisco, California), N1: High-Risk Hereditary Breast Cancers, N2: High-Risk Hereditary Colon Cancers, N3: Hereditary Colon Cancer, N4: Women's Hereditary Cancer, N5: Hereditary Cancer, N6: Hereditary Pancreatic Cancer Panel; O, Johns Hopkins (Baltimore, Maryland); P, University of Pittsburgh Medical Center (Pittsburgh, Pennsylvania), P1: ThyroSeq, P2: Solid Tumors; Q, Emory University (Atlanta, Georgia) – Snapshot; R, Illumina (San Diego, California), R1: TruSight Cancer, R2: TruSeq Amplicon Cancer, R3: TruSight Tumor; S, PathGroup (Brentwood, Tennessee), S1: NGS for solid tumors, S2: SmartGenomics Heme Gene List.

[a] Inclusion in the Cancer Genomics Resource List (CGRL) does not represent endorsement of a test by the College of American Pathologists. In addition, the CGRL is purely descriptive and is not intended to provide consensus or expert opinion regarding the relative value of any given test or gene. The CGRL is not intended to replace the detailed annotations required for test interpretation and/or patient management.

[b] This table represents data as of early May 2014. The panels offered by individual institutions are expected to change over time. Please check with the institution for the most up-to-date offerings.

[c] This column calculates all the panels which the gene is listed in, not the number of institutions.

Table 1. Extended, Continued

Gene	No. of Panels in Which Gene Is Listed[c]	Helpful Refs	Therapeutic, Diagnostic, Prognostic Target (Yes, No, Unknown)	Notes
LTK	1		Unk, Unk, Unk	
MAP2K4	5		Unk, Unk, Unk	
MAP3K1	2	Ellis et al,[149] 2012	Unk, Unk, Unk	
MET	22	Ghiso et al,[162] 2013	Unk, No, No	Therapeutic–nonselective and selective tyrosine kinase inhibitor; antihepatocyte growth factor monoclonal antibodies
MLL3	1		Unk, Unk, Unk	
MYB	2		Unk, Unk, Unk	
NBN	8		Unk, Unk, Unk	
NF1	12		Unk, Unk, Unk	
NOTCH1	21		Unk, No, Unk	Notch inhibitors are in early stages of evaluation; therapeutic–potential; prognostic–chronic lymphocytic leukemia conflicting data
PDGFRA	20	Cassier et al,[157] 2012	Unk, Unk, Unk	
PDGFRB	5		Unk, Unk, Unk	
PIK3CA	24	Banerji et al,[151] 2012; Cancer Genome Atlas Network,[18] 2012	Yes, No, No	
PIK3CB	1	Crowder et al,[164] 2009	Unk, Unk, Unk	
PIK3R1	8		Unk, Unk, Unk	
PTEN	36		Unk, Unk, Unk	
PTPRD	3		Unk, Unk, Unk	
RAD50	5	Weigman et al,[158] 2012	Unk, No, No	Therapeutic–potentially yes; potentially sensitive to PARP inhibitors
RAD51C	6		Unk, Unk, Unk	
RB1	17		Unk, Unk, Unk	
ROS1	6		Unk, Unk, Unk	
RUNX1	12		Unk, Unk, Unk	
SF3B1	7		Unk, Unk, Unk	
SOCS1	4	Zhang et al,[163] 2012	No, No, No	
SYK	2	Kanwal et al,[161] 2012	Yes, Yes, Yes	Risk factor
SYK	2	Kanwal et al,[161] 2012	Yes, Yes, Yes	Risk factor
TLR4	1		Unk, Unk, Unk	
TNFAIP3	5	Vendrell et al,[159] 2007	No, No, No	Overexpression associated with tamoxifen resistance
TP53	39	Banerji et al,[151] 2012; Cancer Genome Atlas Network,[18] 2012	No, Unk, Yes	Diagnostic–maybe

Genomic data can be acquired via many methods, including classical cytogenetics, in situ hybridization, and microarray techniques. In addition, data supporting personalized medicine can be obtained by methods that might not be considered "genomic" per se, such as immunohistochemical staining.[10] The revolution in genomic medicine, however, has largely been driven by the development of next-generation sequencing (NGS) technologies. Several distinctive characteristics have made NGS a prominent technique in contemporary genomic medicine:

1. The massively parallel nature of NGS allows large amounts of sequence data to be acquired simultaneously and at a low cost per nucleotide.
2. Next-generation sequencing is robust to specimen deficiencies, such as DNA degradation, compared with prior techniques.[11]
3. By producing many sequence reads over a given region, NGS is highly sensitive and allows the detection of rare alleles present within a mixed cellular population.[12]
4. The cost of NGS testing scales less than linearly with the size of the assay: the costs of sequencing 50 and 500 genes with NGS differ by less than a factor of 10. Therefore, the incremental cost of adding new genes to a gene set is low.
5. Next-generation sequencing lends itself to a wide range of methodologic adaptations that allow variations in the assay size (whole-genome sequencing versus enrichment methods), the analyte detected (DNA versus messenger RNA versus epigenetic changes), and the types of variants that can be detected (single-nucleotide variants, insertions and deletions, copy number variants, and structural variants).[13]

Table 1. Extended, Continued

Gene	A	B	C	D	E1	E2	E3	E4	F	G	H1	H2	H3	H4	I1	I2	J1	J2	J3	J4	J5	J6	J7	J8
LTK	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MAP2K4	N	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MAP3K1	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MET	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	N	N	N	Y	N	N	N	N	N	Y	N	N
MLL3	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MYB	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
NBN	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	N	Y	N	N	N
NF1	N	Y	Y	Y	Y	N	N	N	N	Y	N	Y	N	Y	N	N	Y	N	Y	N	Y	N	N	Y
NOTCH1	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	N	N	N	N	N	N	N
PDGFRA	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N	Y	Y	Y	N	N	N	N	N	N	N	N
PDGFRB	N	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PIK3CA	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	N	N	N	N	N
PIK3CB	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PIK3R1	N	Y	Y	Y	Y	N	N	N	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N
PTEN	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N
PTPRD	N	N	N	N	Y	N	N	N	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N
RAD50	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	N	Y	N	N	N
RAD51C	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	Y	N	Y	N	N	N
RB1	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	Y	N	N	N	Y	N	N	N	N	N	N	N	N
ROS1	N	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	N	Y	N	N	N	N	N	N	N	N	N
RUNX1	Y	Y	Y	Y	Y	N	N	Y	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
SF3B1	N	Y	Y	N	Y	N	N	N	N	Y	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
SOCS1	N	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SYK	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SYK	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TLR4	N	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TNFAIP3	N	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TP53	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N

In current clinical practice, most NGS cancer testing involves sequencing a panel or set of genes, rather than the entire genome or exome. Sequencing entire genomes or exomes produces a large volume of data without known clinical significance. By focusing on a gene set of interest, a test can achieve greater depth of coverage over regions of interest while minimizing the total amount of sequencing performed. This depth of coverage is particularly critical for cancer testing because of tissue and tumor heterogeneity: somatic variants of interest may be present at a low allele frequency, whereas in most constitutional testing, variants are present at an allele frequency of either 50% or 100%.

Genomic sequencing for cancer care is now offered by commercial for-profit laboratories, independent laboratory networks, and clinical laboratories in academic medical centers and integrated health care networks. There are considerable differences between the tests offered by these early adopters:

1. Some tests are custom-built by the laboratory, while others are based on a vendor-designed kit.
2. Some tests are organ-specific (eg, a panel focusing on lung cancer), while others are relevant to multiple different organs.
3. Different sequencing platforms are used (with most tests using technology from either Illumina [San Diego, California] or Ion Torrent [Life Technologies, Carlsbad, California]).
4. The tests also differ in enrichment methodology, the major distinction being hybrid capture versus amplification.

Table 1. Extended, Continued

Gene	K	L1	L2	L3	M1	M2	N1	N2	N3	N4	N5	N6	O	P1	P2	Q	R1	R2	R3	S1	S2
LTK	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MAP2K4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MAP3K1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MET	Y	Y	N	N	N	Y	N	N	N	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	N
MLL3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MYB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
NBN	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N
NF1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N
NOTCH1	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	N	N	Y
PDGFRA	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	N	Y	Y	Y	Y
PDGFRB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PIK3CA	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	Y	N	Y	Y	Y	Y
PIK3CB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PIK3R1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N
PTEN	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	N	Y
PTPRD	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
RAD50	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
RAD51C	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N
RB1	Y	Y	N	N	N	Y	N	N	N	N	N	N	Y	N	Y	N	Y	Y	N	N	N
ROS1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
RUNX1	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	Y	Y
SF3B1	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
SOCS1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SYK	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SYK	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TLR4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TNFAIP3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
TP53	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	Y

The variability among the currently available tests has created a complex environment, which may be confusing to clinicians and pathologists as both users and as developers of NGS cancer testing. To address this complexity, in 2009, the Personalized Health Care (PHC) Committee of the College of American Pathologists (CAP) proposed the development and synthesis of a cancer genomics resource list. The goal was to develop a list of cancer gene panels offered by early-adopter academic and commercial laboratories operating in CAP-accredited, Clinical Laboratory Improvement Amendments of 1988 (CLIA)–certified environments. The PHC Committee established the Core Cancer Panel Working Group in 2012 to address this goal. The present article, including the accompanying Table 1 representing breast cancer genetic mutations and associated Web site, is the product of that working group's efforts.

METHODS

Members of the working group were selected by the chair for their expertise in NGS testing and their knowledge of particular disease genes. The task force included a specialist in each of the major organ systems.

The working group members used their professional knowledge to identify academic pathology departments and reference laboratories offering NGS testing in the fall of 2013. One large community-based practice was included. Each laboratory was queried to determine the genes included in the test or tests offered. These gene lists were posted on some laboratories' Web sites; in other cases, they were provided by laboratory directors. When information was obtained via a Web site, the vendor or organization was contacted to confirm the information's accuracy and timeliness. The gene lists were periodically reconfirmed with the laboratories, and any changes were incorporated (last update May 5, 2014).

Table 2. Data Elements Abstracted for Gene-Disease Pairs
Organ system
Specific tumor type
Mutation type and position
Expected mutation frequency
Significance (diagnostic, therapeutic, prognostic)
Associated drug, for therapeutically significant genes
References
Number of panels in which gene is listed

The gene lists offered by these early adopters were compiled into a table (Table 1; and supplemental digital content including Supplemental Tables 1 and 2, available for this article at www.archivesofpathology.org in the August 2015 table of contents) without additions or deletions. The genes were then annotated by 1 or more working group members. Specifically, each member reviewed the table to identify genes relevant to his or her assigned organ system. Because a single gene may have different effects in different diseases, the working group created a separate row for each annotated gene-disease pair. The annotation process drew on published knowledge, including public databases and the medical literature. The data elements extracted for each gene-disease pair are listed in Table 2. Not all elements were abstracted for all gene-disease pairs. In addition, genes that were present in only 1 or 2 panels were considered to have insufficient uptake to justify the effort required to annotate them. These are listed in Supplemental Table 2.

The working group chair reviewed the table as a whole for quality assurance. The final product, henceforth referred to as the Cancer Genomics Resource List 2014 (CGRL), is available as supplemental digital content at www.archivesofpathology.org in the August 2015 table of contents. A Table of only those genes offered for breast cancer mutation testing is included in the article as Table 1. The Resource List was current as of May 5, 2014; however, the working group recognizes that all the elements of the table will change over time. Inclusion in the CGRL does not represent endorsement of a test by the CAP. In addition, the CGRL is purely descriptive and is not intended to provide consensus or expert opinion regarding the relative value of any given test or gene. The CGRL is not intended to replace the detailed annotations required for test interpretation and/or patient management. The references were derived from a number of sources, including the following CAP Pathology Resource Guides: Genomic Analysis and Molecular Diagnosis, CAP SPECs (Short Presentations in Emerging Concepts) references, and journal articles.

RESULTS

The working group consisted of site-specific experts in cancer genetic sequencing and early adopters of NGS technology. This group identified current NGS-based cancer tests for inclusion in the CGRL (Table 3).

The gene lists obtained from these laboratories and vendors, accompanied by the annotations produced as described in "Methods," were aggregated into the CGRL (available as Cancer Genomics Resource List 2014). The CGRL can be sorted according to any column. Some users may choose to sort by organ system or tumor type: this view provides a useful reference for those interested primarily in a specific subset of cancers (Figure 1). Sorting by gene is also particularly useful for easily assessing the known significance of a given gene across tumor types.

The number of genes per panel ranged from 6 (Ambry Genetics' [Aliso Viejo, California] BRCAplus and Invitae's [San Francisco, California] High-Risk Hereditary Breast Cancers) to 408 (Life Technologies' Ion AmpliSeq Comprehensive Cancer Panel) (Figure 2). The CGRL includes 611 different genes for which NGS-based mutation testing is offered. Surprisingly, of these 611 genes, 0 genes were listed in every panel, 43 genes were listed in 4 panels, and 54 genes were listed in 3 panels (Figure 3). In addition, tests for 393 genes were offered by only 1 or 2 institutions. Mutations in these genes are quite rare. The working group annotated the 218 genes that were included in 3 or more panels and chose not to annotate the remaining genes (Figure 4).

Table 3. Laboratory-Developed and Vendor-Developed Panels Included in the Cancer Genomics Resource List 2014

Laboratory/Vendor	Test Name
Washington University (St Louis, Missouri)	Comprehensive Cancer Gene Set, v2
Foundation Medicine (Cambridge, Massachusetts)	FoundationOne
Brigham and Women's Hospital (Boston, Massachusetts)	
Qiagen (Germantown, Maryland)	GeneRead DNAseq Human Comprehensive Cancer Panel
Life Technologies (Carlsbad, California)	Ion AmpliSeq Comprehensive Cancer Panel, Ion AmpliSeq Cancer Hotspot Panel v2, Ion AmpliSeq Colon and Lung Cancer Panel, Ion AmpliSeqAML Panel
Baylor College of Medicine (Houston, Texas)	
University of Washington (Seattle, Washington)	Oncoplex Panel
Knight Diagnostic Laboratories (Portland, Oregon)	GeneTrails NSCLC Genotyping Panel, GeneTrails Solid Tumor Panel, GeneTrails AML MDS Genotyping Panel, GeneTrails GIST Genotyping Panel
Caris Life Sciences (Irving, Texas)	Solid Tumors, Next-Generation Sequencing
Ambry Genetics (Aliso Viejo, California)	CancerNext, *BRCA*Plus, BreastNext, ColoNext, OvaNext, RenalNext, PancNext, PGLNext
ARUP Laboratories (Salt Lake City, Utah)	Solid Tumor
Montefiore Medical Center (Bronx, New York)	Hot Spot, other somatic genes, other germline genes
Mayo Medical Laboratories (Rochester, Minnesota)	Colorectal Cancer Panel, 50-Gene Panel
Invitae (San Francisco, California)	High-Risk Hereditary Breast Cancers, High-Risk Hereditary Colon Cancers, Hereditary Colon Cancer, Women's Hereditary Cancer, Hereditary Cancer, Hereditary Pancreatic Cancer Panel
Johns Hopkins University (Baltimore, Maryland)	
University of Pittsburgh (Pennsylvania)	ThyroSeq, Solid Tumor
Emory University (Atlanta, Georgia)	Snapshot
Illumina (San Diego, California)	TruSight Cancer, TruSeq Amplicon Cancer, TruSight Tumor
PathGroup (Brentwood, Tennessee)	NGS (Next-Generation Sequencing) for Solid Tumors, SmartGenomics Heme Gene List

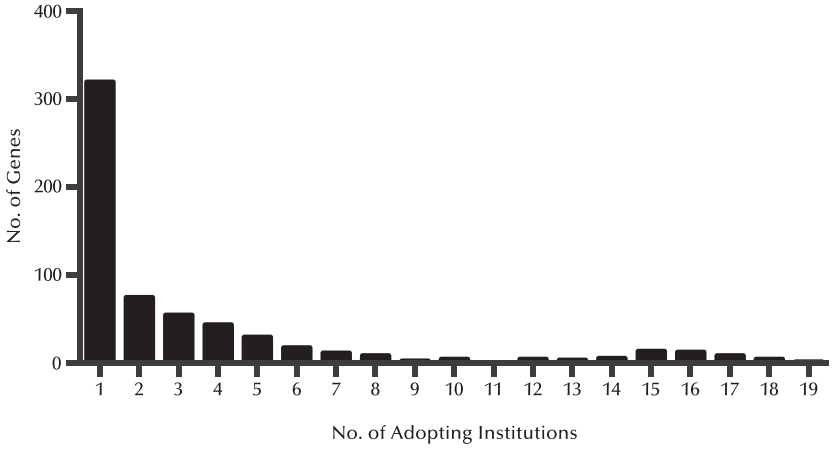

Figure 1. *Number of institutions offering gene panels containing from 1 to more than 300 genes. Each bar shows the number of genes that were offered by the stated number of laboratories.*

Breast Cancer

Breast cancer is not a single disease; rather, its molecular and clinical characteristics vary substantially.[14,15] Gene expression profiling has been used to identify early breast cancer cases with such a low risk of recurrence after local therapy and antihormonal therapy that the addition of adjuvant chemotherapy would provide little benefit.[16,17] The Cancer Genome Atlas (TCGA) recently performed a comprehensive molecular analysis of more than 500 breast cancers; only 3 genes (*TP53*, *PIK3CA*, and *GATA3*) mutated in more than 10% of the tumors were identified. Commonly altered genes included *TP53*, *PIK3CA*/*PTEN*, and *RB1*; however, the mechanisms of alteration were quite variable and included mutations, deletions, amplifications, and copy number alterations.[18] The Molecular Taxonomy of Breast Cancer International Consortium assessed 2000 cases of breast cancer and identified 10 subgroups with unique gene expression, genomic profiles, and clinical outcomes, demonstrating that the frequency of mutations was variable among the different subgroups.[19,20] Although these findings are not yet actionable, it is hoped that they will facilitate a more personalized approach to breast cancer in the near future. Table 1 provides a detailed list and the annotation of the genes associated with breast cancer included in the CGRL.

Gastrointestinal—Gastric Cancer

Gastric cancers carry numerous genomic aberrations that result in the development and progression of carcinogenesis.[21,22] However, few genomic alterations currently have demonstrated clinical value. Genomic alterations in gastric cancers that have been validated or are in advanced stages of evaluation for clinical application include *HER2*/*neu* expression and amplification as well as *MET* and *FGFR* amplification.

A randomized phase 3 clinical trial (ToGA trial) established the utility of targeting *HER2/neu* in advanced gastric cancer[23] by demonstrating that metastatic gastric or gastroesophageal adenocarcinomas positive for *HER2/neu* amplification or overexpression showed improved overall survival in trastuzumab-treated subjects. Testing *HER2/neu* expression and amplification status and adding trastuzumab to the chemotherapy regimen for advanced *HER2/neu*-positive gastric and esophageal cancers has become the standard of care. The overexpression and amplification of *HER2/neu* has been described in 6% to 35% of gastric and gastroesophageal junction adenocarcinomas.[24,25] *HER2/neu* amplification in gastric carcinoma is associated with poor outcome[25–27] and has been shown to be an independent prognostic factor.[24] The CAP reviewed the current guide-

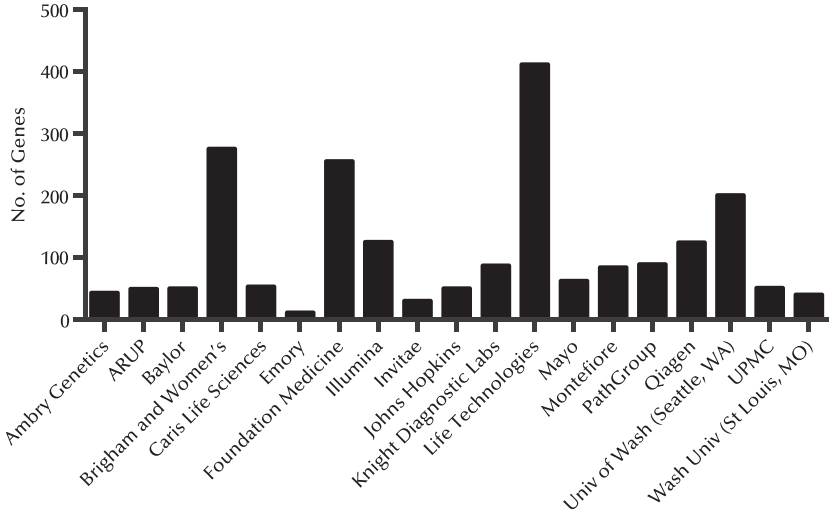

Figure 2. *Number of genes offered by each institution. The institutions include the following: Ambry Genetics, Aliso Viejo, California; ARUP Laboratories, Salt Lake City, Utah; Baylor College of Medicine, Houston, Texas; Brigham and Women's Hospital, Boston, Massachusetts; Caris Life Sciences, Irving, Texas; Emory University, Atlanta, Georgia; Foundation Medicine, Cambridge, Massachusetts; Illumina, San Diego, California; Invitae, San Francisco, California; Johns Hopkins University, Baltimore, Maryland; Knight Diagnostic Laboratories, Portland, Oregon; Life Technologies, Carlsbad, California; Mayo Medical Laboratories, Rochester, Minnesota; Montefiore Medical Center, Bronx, New York; PathGroup, Brentwood, Tennessee; Qiagen, Germantown, Maryland; University of Washington, Seattle, Washington; University of Pittsburgh Medical Center (UPMC), Pittsburgh, Pennsylvania; and Washington University, St Louis, Missouri. Abbreviations: Labs, Laboratories; Univ, University.*

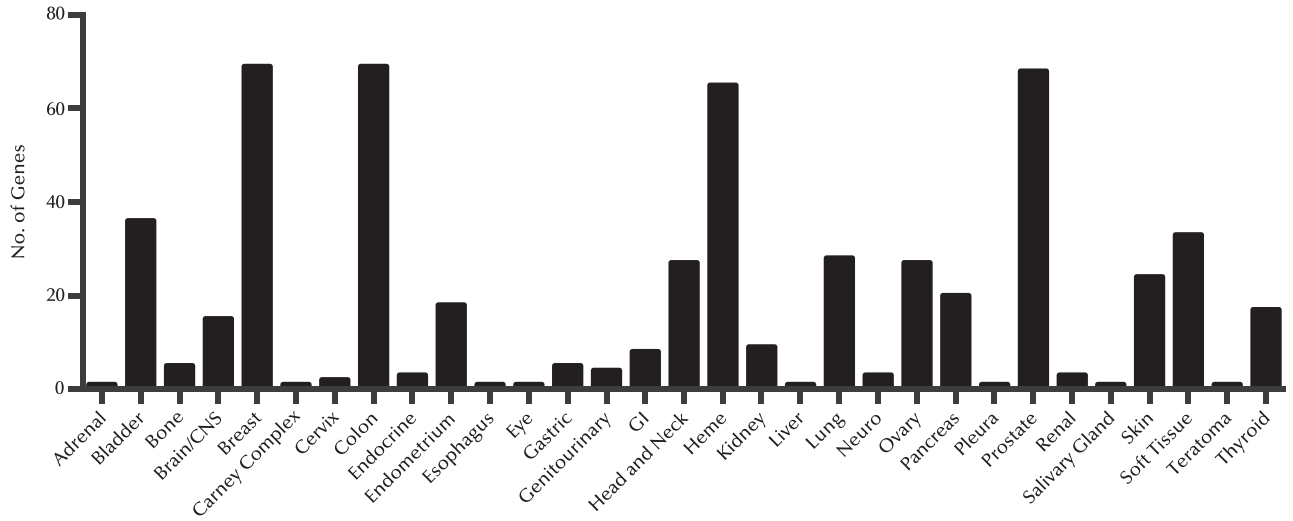

Figure 3. *Number of genes annotated as being clinically significant at various disease sites. Abbreviations: CNS, central nervous system; GI, gastrointestinal; Heme, hematologic; Neuro; neurological.*

lines for the interpretation of *HER2/neu* expression and amplification.[23,27,28]

Other promising molecular targets in advanced gastric cancer include the overexpression and amplification of the *MET* and *FGF* receptors. MET- and FGFR-targeted therapies are being evaluated in phase 3 trials. *MET* amplification defines a small (2% of tested cases) but aggressive subset of gastric and esophageal adenocarcinomas, suggesting sensitivity to the targeted *MET* inhibitor crizotinib.[29] *FGFR2* gene amplification, which was detected in 2% of tested gastric tumors, predicts sensitivity to the selective fibroblast growth factor receptor (FGFR) inhibitor AZD4547.[30]

In contrast to colorectal cancer, anti–epidermal growth factor receptor (EGFR) therapies (cetuximab, panitumumab, and gefitinib) failed to improve the outcomes of gastric cancer patients in phase 3 trials.[31] In addition, *EGFR* pathway gene mutations failed to predict resistance to anti-EGFR targeted therapies.[32]

Gastrointestinal—Colorectal Cancer

The most important driver genes of colorectal carcinogenesis have been identified, although the systematic incorporation of this information into a set of clinically useful markers for prognosis or the prediction of susceptibility to therapeutic agents has proven elusive.[33,34] However, a recent review of mutations identified by the TCGA colorectal cancer study found a high incidence (70%) of cancers with potentially "druggable" mutations.[35] The most important mutations linked to known therapeutic benefits in colon cancer were in exons 2, 3, and 4 of *KRAS* and *NRAS*. The absence of a *KRAS* or *NRAS* mutation implies an intact EGFR-MAPK pathway that is potentially susceptible to anti-EGFR therapy. A therapeutic response generally increases survival by only a few months on average.[36] A *BRAF* mutation (V600E) in microsatellite-stable colon cancer is associated with a poor outcome but is not a universally accepted predictive marker of anti-EGFR therapy.[37]

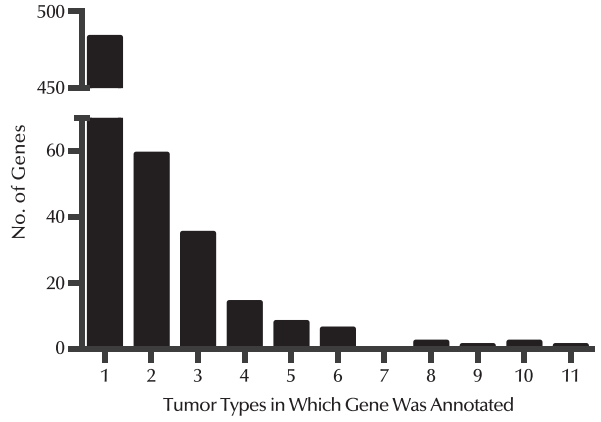

Figure 4. *Number of genes relevant in multiple tumor types.*

Gastrointestinal—Pancreatic Cancer

Numerous genetic alterations are involved in the molecular carcinogenesis of pancreatic adenocarcinoma. Genes that are frequently mutated in pancreatic cancer include *KRAS*, *CDKN2A* (p16), *TP53*, and *SMAD4*/*DPC4*.[37] In addition to these key genes, numerous other genetic mutations have been identified that impact various cellular pathways involved in pancreatic carcinogenesis and can potentially serve as theranostic targets. Some of the mutated genes that have been identified in pancreatic cancer and have been added to clinical testing panels include *MLL3*, *TGFBR2*, *ARID1A*, *SF3B1*, *ATM*, and *ARID2*.[38] The *GATA4* gene product was found to be overexpressed by immunohistochemical testing of pancreatic ductal adenocarcinoma.[39] Two genes, *KLF6* and *NFKBIZ*, were identified as part of a 6-gene panel used to predict the survival of patients with localized pancreatic ductal adenocarcinoma; both of these genes showed higher expression in the poor prognosis group.[40] Immunohistochemical analyses revealed that elevated tumor expression of the proteins encoded by 2 genes, *ERCC1* and *RRM1*, was associated with poor prognosis[41]; these genes may serve as potential biomarkers for personalized chemotherapy. Studies of patients with pancreatic cancer treated with gemcitabine have suggested that tumors with high RRM1 expression portended a better prognosis than those with low RRM1 expression,[42] and ovarian cancer studies have suggested that tumors with high ERCC1 expression had a poor response to platinum-based thera-

py.[43] Although some of the genes mentioned above appear to have prognostic utility, none of these genes or their protein products are currently useful in the diagnosis of pancreatic adenocarcinoma, nor are they currently approved as theranostic markers.

Intraductal papillary mucinous neoplasms (IPMNs) can give rise to pancreatic adenocarcinoma; however, these cystic lesions are often difficult to diagnose preoperatively. Three genes included in clinical testing panels may be of diagnostic utility. Inactivating mutations in *STK11*, a tumor suppressor gene, have been identified in IPMNs but not in another known mucinous precursor lesion, pancreatic intraepithelial neoplasia.[44] Activating mutations in *PIK3CA*, which have been found in a minority of IPMNs, may identify a specific variant called intraductal tubulopapillary neoplasm.[45] Finally, more than 40% of IPMNs harbor mutations in *GNAS* (and often in *KRAS* as well), a feature that can be of diagnostic utility in preoperative cyst fluid analyses[46] because other cystic pancreatic lesions do not appear to harbor *GNAS* mutations.[44]

Genitourinary—Prostate Cancer

Approximately 50% of all primary prostate cancers possess *TMPRSS2:ERG* rearrangements.[47] Several other *ERG* rearrangements have been reported in prostate cancer, including rearrangements involving the ETS family members *ETV1* (7p21.2) and *ETV4* (17q21) as well as the *RAF* kinase gene.[48–52] The subsequent overexpression of the ERG protein is virtually 100% specific for the presence of prostate cancer in surgical specimens.[53,54] *SPOP* is the most frequently mutated gene in prostate cancer studies, with mutations found in 6% to 15% of cases across multiple independent cohorts.[55,56] Recurrent genetic alterations in prostate cancer frequently involve genes that mediate *AR* signaling, common tumor suppressors, and chromatin modification genes. The activation of the PI3K and RAS/RAF signaling pathways occurs in approximately 42% of prostate cancers.[57] *SPINK1* mutations define an aggressive molecular subtype of *ETS* fusion–negative prostate cancers and account for approximately 10% of all prostate cancers.[58] *EZH2* is significantly upregulated in prostate cancer and is associated with cancer aggressiveness.[59] Other genetic alterations associated with prostate cancer include the loss of *NKX3.1* and *PTEN* as well as mutations in *AR*, *AKT1*, *TP53*, *RB1*, and *PIK3CA*.[55,57,60,61]

Genitourinary—Bladder Cancer

The most important driver mutations of urothelial bladder carcinoma occur in *FGFR3* and *TP53*, which define 2 distinct pathways in urothelial bladder carcinoma.[62] *FGFR3* mutations are associated with low-stage, low-grade, superficial papillary tumors with favorable disease characteristics and lower rates of recurrence and progression; *TP53* mutations occur in up to 70% of muscle-invasive bladder cancers and are associated with high-stage, high-grade tumors.[63] Carcinoma in situ has a high rate of *TP53* mutations,[64] and the following genetic mutation rates have been reported for urothelial carcinoma: *PIK3CA*, 26%; *CDKN2A/B*, 23%; *RB1*, 17%; *CCND1*, 14%; *FGFR1*, 14%; *CCND3*, 11%; *FGFR3*, 11%; *MDM2*, 11%; *EGFR*, 6%; and *HER2/neu*, 6%.[65] Other genetic alterations have been identified in bladder carcinogenesis, including mutations in *RAS* gene family members and in *TERT*, as well as the loss of the *RB1* and *PTEN* tumor suppressors.[63,66–68] With the exception of genes such as *FGFR3*, *TP53*, *RB1*, *HER2/neu*, *CDKN2A*, *VEGF*, and *PTEN*, most of the identified alterations have not been related to outcome or treatment response; thus, their roles require further refinement.[60,63]

Gynecologic Cancers

The genetic tests identified by this working group included approximately 40 genes with specific relevance to gynecologic cancers, particularly endometrial and ovarian cancers. Many of these genes have functions in multiple tumor types and sites; for example, the tumor suppressor gene *PTEN* is commonly mutated in endometrioid cancers of both the endometrium and ovary. Several important genes in gynecologic cancers—for example, *PTEN*, *ARID1A*, and *HER2/neu*—may exhibit somatic mutations in numerous regions of the gene, making a sequencing-based approach more attractive than hotspot testing or single-nucleotide genotyping. Several targeted therapies for gynecologic cancers are either in current use or in advanced stages of development, including tyrosine kinase inhibitors for *FGFR2*-mutated tumors, PARP inhibitors[69–71] for tumors with "BRCAness,"[72,73] and mTor inhibitors for tumors with mutations in the PI3K/mTor/Akt pathway.[74–76] Next-generation sequencing testing is therefore positioned to play an important role in therapy selection for cancers of this organ system.

Head and Neck Cancers

Most head and neck cancers are squamous cell carcinomas, which are often associated with human papillomavirus (HPV) infection or with alcohol and tobacco use.[77] Head and neck squamous cell carcinomas (HNSCCs) are heterogeneous tumors with mutational patterns that differ between HPV-positive and HPV-negative tumors.[78] The mutational rate of non–HPV-related HNSCCs is comparable to that of other smoking-related malignancies and is approximately double that of HPV-related HNSCCs.[78] Recently, molecular subtypes of HNSCCs were identified by using expression profiling.[79] Most cases of HNSCC exhibit high EGFR expression due to amplification (approximately 17% of cases) or posttranslational modifications. Genetic alterations in the PIK3/AKT/mTOR pathway, which is downstream of EGFR, are also often identified in HNSCCs (74% of cases).[80] *PIK3CA* is of particular interest because inhibitors are already under development for breast cancer treatment. Several salivary gland neoplasms and HNSCCs are associated with translocations, including *MECT1/MAML* t(11;19) in mucoepidermoid carcinoma, *MYB/NFIB* t(6;9) in adenoid cystic carcinoma, *ETV6/NTRK3* t(12;15) in mammary analog secretory carcinoma, and *EWSR/ATF1* t(12;22) in clear cell carcinoma. As research in the role of genetic translocations in head and neck cancers continues to develop, additional significant genes are likely to be identified.

Head and Neck—Thyroid Cancer

Thyroid cancer is the most common malignancy of endocrine organs, and its incidence is increasing steadily in the United States and worldwide. It is clinically important to establish a thyroid cancer diagnosis preoperatively and to separate thyroid nodules that are malignant and need to be surgically removed from benign nodules that do not require surgery. It is also important to predict aggressive behavior of well-differentiated thyroid cancers and to select appropriate therapies for dedifferentiated tumors. Performing NGS of genes involved in thyroid carcinogenesis can address these challenges.[81] The most common mutations in papillary

thyroid cancer (PTC) are point mutations of the *BRAF* and *RAS* genes and rearrangements of *RET*/*PTC*, *NTRK1*, and *NTRK3* (a recent discovery), all of which are able to activate the mitogen-activated protein kinase (MAPK) pathway. These mutations are found in more than 70% of PTCs.[81–85] *RAS* mutations and *PAX8*/*PPARGγ* rearrangements, which are also mutually exclusive, have been identified in 70% to 75% of follicular thyroid carcinomas.[81,86] Genetic alterations involving the PI3K/AKT signaling pathway have also been observed in thyroid tumors, particularly in advanced and dedifferentiated tumors.[81,87–89] Additional mutations known to occur in poorly differentiated and anaplastic carcinomas involve the *TP53*, *AKT1*, and *CTNNB1* genes. Medullary thyroid carcinomas, both familial and sporadic, frequently carry point mutations in the *RET* and *RAS* genes.[81,90,91] All of these genetic alterations are helpful for establishing a diagnosis of malignancy in thyroid fine-needle aspiration (FNA) samples.[81] In addition, mutations in some genes (eg, *BRAF* and *TERT*) are associated with a more aggressive tumor phenotype and can be used as prognostic markers.[92,93] Finally, a number of known (*BRAF*, *RET*) and recently discovered (*STRN-ALK*) mutational markers may guide the selection of targeted therapies for aggressive types of thyroid cancer.[94]

Hematologic Malignancies

Many clinically relevant and disease-dependent alterations in hematologic malignancies are translocations. In acute myeloid leukemias and myeloproliferative neoplasms, the presence of a specific translocation determines malignancy and is used to identify the category and subtype of leukemia, as outlined by the *World Health Organization Classification of Tumours*.[95] For example, a translocation such as the Philadelphia chromosome, t(9;22)(q34;q11), which involves the *BCR* and *ABL* genes, is required for diagnosis of chronic myelogenous leukemia. In addition to diagnostic information, identifying the *BCR*/*ABL* translocation provides important information about the patient's prognosis and response to the *ABL* kinase-specific drug imatinib. A diagnosis of acute promyelocytic leukemia requires the identification of the t(15;17)(q24:q12); *PML*/*RARA* rearrangement or related rearrangements. The response to trans retinoic acid and anthracycline depends on which specific translocation is present. The clinically relevant translocations involved in all the subtypes of myeloid neoplasms are too numerous to describe here.

Myeloid malignancies are also associated with many prognostically and therapeutically important inactivating and activating mutations, short deletions, and duplications that affect the clinical course and can be identified by using NGS. These include alterations in *FLT3*, *NPM1*, *CEBPA*, *c-KIT*, *IDH1*, *IDH2*, *TET2*, *MLL*, *DNMT3A*, *JAK2*, *RUNX1*, and many more.[96]

Translocations are a diagnostic and prognostic feature of many lymphoid malignancies of mature and immature T and B cells. Translocations involving the immunoglobulin heavy-chain or κ or λ light-chain genes, on chromosome 14, 2, and 22, respectively, are observed in B-cell malignancies. Furthermore, translocations involving the T-cell receptor α, β, λ, or δ genes, on chromosomes 14(q11), 7(q35-6), 7(p15), and 14(q11), respectively, are observed in T-cell malignancies. The translocation partners are extremely varied and are either diagnostic or prognostic in many cases. Many other partners are classically found in B-cell and T-cell malignancies. The hallmark translocations t(8;14)(q24;32), t(14;16)(q32;q21), and t(11;14)(q13;32) are associated with Burkitt lymphoma, follicular lymphoma, and mantle cell lymphoma, respectively. Single-nucleotide variants are not commonly found in malignant lymphomas.[95,97]

Translocations are frequently used as diagnostic and prognostic markers for different subtypes of precursor B and T lymphoblastic leukemia/lymphoblastic lymphoma. The following translocations are observed in precursor B lymphoblastic leukemia/lymphoma: t(12;21)(p13;q22) involving *TEL*/*AML1* (16%–29% of cases), t(1;19)(q23;p13.3) involving *PBX/E2A* (6% of cases), t(4;11)(q21;q23) involving *AF4*/*MLL* (2%–3% of cases), and t(9;22)(q34;q11.2) involving *BCR*/*ABL* (3%–4% of cases). Each translocation confers a different clinical phenotype and prognosis, and clinical decisions are influenced by the identification of these specific abnormalities. Mutations in *CRLF2*, *IKZL1*, *TP53*, *ERG*, and *NOTCH* have also been detected in precursor B and T lymphoblastic leukemia/lymphoblastic lymphoma and confer an adverse prognosis.[96,97] Many additional translocations have been identified in patients with T lymphoblastic leukemia/lymphomas, but these have not been linked to diagnostic subtype, prognosis, or therapy as abnormalities in precursor B lymphoblastic leukemia/lymphoblastic lymphoma have.

In contrast to lymphomas, mature B-cell lymphoid malignancies, chronic lymphocytic leukemia (CLL), and hairy cell leukemia are associated with characteristic deletions, insertions, chromosome duplications, and mutations, which define disease progression and clinical prognosis. The most common aberrations in CLL include 13q deletion, trisomy 12, and deletions of 11q, 17p, or 6q. Many other genes are commonly mutated in CLL, including the following: *NOTCH1*; *XPO1* (exportin 1); *MYD88*; *KLH6* (Kelch-like 6); *TP53*; *TGM*; *BIRC3*; *PLEKHG5*; *ATM*; *SF3B1* (splicing factor 3, B1 unit); *ZMYM3*; *MAPK1*; *FBXW7*; and *DDX3X*. Mutations in these genes often confer a worse prognosis.[98–100]

The identification of the *BRAF* V600E mutation in almost 100% of hairy cell leukemia cases has refined the diagnosis, prognosis, and therapeutic strategies for this disease.[101] This mutation is found only rarely in multiple myeloma or other B-cell malignancies.

Lung Cancer

Lung adenocarcinoma is one of the highest profile cancers for which NGS is beneficial. Several critical, mutually exclusive "driver" oncogenes have been found to render some lung cancers susceptible to treatment with specific targeted inhibitors. The first discovered and largest (~20%) group of lung adenocarcinomas carry mutations in exons 18 to 21 of *EGFR*. Among these tumors, approximately 80% respond radiographically to treatment with targeted inhibitors of the *EGFR* tyrosine kinase domain (TKIs), such as gefitinib or erlotinib, with a resulting increase in progression-free survival in advanced-stage lung cancer from approximately 6 months to approximately 15 months. However, these patients inevitably relapse, often with either a secondary mutation in *EGFR* exon 20 or with a copy number gain of the *MET* oncogene, although other mechanisms have been reported. Following the discovery of *EGFR*, similar responses were reported for tumors that contained *ALK* (~5%) or *ROS1* (~1%) rearrangements and were treated with an *ALK* TKI. Other small subsets of patients with lung adenocarcinomas have been reported to bear rearrangements of *RET*, *NTRK1*, or *FGFR1/2/3*, or

mutations involving *HER2/neu*, *BRAF*, or *PIK3CA*; each of these populations is currently being studied for its response to specific targeted inhibitors, with promising preliminary results.

By contrast, less is known concerning squamous cell or small cell carcinomas of the lung. These cancers do not appear to contain the same alterations or exhibit the same responses to targeted therapies as adenocarcinomas. There have been recent reports of squamous cell carcinomas of the lung bearing several recurrent alterations, including copy number gains or, less commonly, mutations in the *FGFR* genes and in *DDR2*. Case reports of responses to targeted inhibitors have been published, and clinical trials are ongoing.

Melanoma

Melanoma can be divided into multiple genetic subsets by mutations that are generally mutually exclusive. The *BRAF* V600 mutation in exon 15 is present in up to half of cutaneous melanoma cases, which can be treated with US Food and Drug Administration–approved inhibitors,[102–106] for which clinical trials are underway to determine the best therapy.[107–109] Both *NRAS* and *BRAF* mutations may also carry prognostic significance.[110–113] Another subset of melanoma cases exhibit a loss of *NF1*, which encodes a RAS-GTPase (guanosine triphosphatase)–activating protein that normally inactivates the wild-type RAS protein; the loss of *NF1* increases the activation of pathways downstream of RAS.[114,115] Up to 20% of mucosal and acral lentiginous melanomas, which are very rare compared with cutaneous cases, possess activating mutations and/or amplification of the *KIT* locus on 4q12.[116,117] Such cases can be treated with the appropriate tyrosine kinase inhibitors.[118] Because *KIT*, *NRAS*, and *NF1* mutations in melanomas tend to activate both the ERK and AKT pathways to some degree, these mutations are generally mutually exclusive.[114,119,120] *BRAF* mutations primarily activate the ERK pathway, but a complementary loss of *PTEN* or other events can concomitantly activate AKT in these tumors.[108] One-third of cutaneous melanomas lack mutations in any of these genes, but many show a variety of genetic events that activate the ERK and AKT pathways. Uveal melanomas adopt an alternative genetic route, with activating mutations in *GNAQ* and *GNA11*, which encode α subunits of heterotrimeric G proteins that activate phospholipase signaling pathways.[121,122] Melanomas commonly contain mutations in other genes, such as *CDKN2A*, *TP53*, and other, more novel genes; however, no targeted therapies have been developed for such cases.[114,119,120,123] Multiplex genetic testing approaches are increasingly used to assign patients to the targeted therapy approaches that are best suited to their specific mutations. Mutational profiles may also facilitate the selection of immune activation treatments, which show great promise in melanoma.[124–126]

Nervous System Tumors

Although there are a wide range of central nervous system tumors, genetic changes are particularly relevant in neuroblastomas and gliomas. *MYCN* amplification is a well-known genetic abnormality in neuroblastoma, but somatic mutations are found in only a small number of tumors.[127,128] *ALK* mutations are the most common somatic mutation in neuroblastoma and represent a potential therapeutic target. In low-grade gliomas, the most relevant genetic changes are the codeletion of 1p and 19q and *IDH1* mutations, both of which are associated with a better prognosis and the oligodendroglial phenotype.[129] Numerous genetic changes have been found in glioblastoma multiforme (GBM). Many of these changes are not unique to GBM but are common in other cancers (*PTEN*, *P53*, *EGFR*, *PDGFRA*, and *PIK3CA*); others, such as *TERT* mutations and *MGMT* promoter methylation, are more specific to GBM.[130] *MGMT* promoter methylation leads to the silencing of *MGMT* and predicts treatment response and prognosis.[131]

Sarcomas

Sarcomas, whether occurring in soft tissues, the gastrointestinal tract, reproductive organs, or elsewhere, are associated with translocations that produce functional fusion genes.[132] Although the presence of a particular translocation is not an absolutely sensitive or specific indicator of a given diagnosis, a limited set of translocations is characteristic of many sarcomas. Consequently, testing for chromosomal rearrangements has been incorporated into sarcoma treatment guidelines.[133] Unfortunately, it is difficult to identify translocations by using chromosomal DNA sequence analyses (either traditional Sanger methods or NGS approaches) because the breakpoints that produce the rearrangements occur within different introns of the involved genes in regions that are at least several kilobases long.[134] Consequently, interphase fluorescence in situ hybridization or reverse transcriptase–polymerase chain reaction are the laboratory methods most commonly used to detect translocations. However, NGS methods can be used to identify translocations with the appropriate "wet-lab" assay design and bioinformatic analysis, most commonly using a hybrid capture approach.[135,136] The development of targeted therapies directed against specific proteins in the intracellular cascades activated by the fusion gene–encoded chimeric proteins has promoted interest in testing for characteristic translocations in sarcomas to guide therapy rather than merely to guide diagnosis.

In addition, single-nucleotide variants (eg, inactivating mutations in *hSNF5*/*INI1*) and short indels (eg, short in-frame deletions in *KIT*) are increasingly recognized as characteristic of other sarcoma types. In contrast with translocations, these mutation types can readily be identified by using both NGS approaches and traditional sequencing methods. For some tumor types (eg, gastrointestinal stromal tumors), specific mutations are correlated with the response to particular therapeutic agents.[137] Furthermore, because the mutations that allow some tumors to escape primary therapy have been categorized, it is sometimes possible to use targeted therapy even in the context of recurrent disease.[137]

COMMENT

The goal of developing this comprehensive list of cancer gene panels offered by early NGS adopters was to assist the laboratory pathology communities. The main audience to which our effort was targeted included the current and next waves of pathologist adopters of targeted large panels for cancer, based on the NGS platform. This list will provide the greatest benefits for academic and large reference laboratories, although large pathology groups and hospital systems will also benefit. The rapid availability of the list will assist in the development of laboratory-developed tests for individual use. Pathologists could design a gene panel by using the data included in the CAP cancer resource list and adding

other genes specific to the interest of their institution. For-profit reference laboratories can use the panel to develop in vitro diagnostic medical device kits, and pharmaceutical companies can use it to develop clinical trials. Finally, both hematologists/oncologists and patients might find the list useful. Therefore, the resource list is expected to benefit current pathology adopters, the next wave of pathology adopters, and all the members of the pathology and molecular diagnostic communities, including providers and patients.

Multigene NGS tests have been described by using various terms, including gene set and panel. The term panel has been criticized by payers and regulators owing to concerns related to test bundling. When clinicians can order multiple separate tests by checking a single box, they may become desensitized to the cost of the individual tests or to the possibility of ordering less than the full panel.[138] Indeed, anecdotal evidence indicates that payers have refused to approve NGS panels on the grounds that they represent unnecessary test bundling. However, we submit that the term *panel* has a special meaning in the NGS context. Instead of representing a grouping of multiple individually orderable tests, a multigene NGS panel represents a single test that encompasses multiple genomic regions. Most critically, eliminating individual genes from an NGS panel does not significantly reduce the cost of the assay. The blanket rejection of these tests on the semantic grounds that they represent "panels" obscures real questions that remain about the optimal design of these tests. We therefore propose the re-adoption of panel as a valid and useful term to describe multigene NGS assays.

In selecting genes for inclusion in clinical genomics assays, some laboratories have chosen to create disease-specific gene sets (eg, "lung genes" for lung cancer), while others have designed a single "pan-cancer" test appropriate for all cancer types. Each of these approaches has advantages and disadvantages.

Among the laboratories surveyed by the CGRL Working Group, Knight Diagnostic Laboratories at Oregon Health and Science University (Portland)[139] exemplifies the disease-specific approach; this laboratory offers a specific NGS test for non–small cell lung cancer, another for gastrointestinal stromal tumors, and another for leukemia. Similarly, the University of Pittsburgh Medical Center (Pittsburgh, Pennsylvania) has developed a specific panel for the preoperative diagnosis of thyroid cancer.[81] Ambry Genetics has also created a series of cancer site-specific NGS tests,[140] although these tests are designed to detect constitutional cancer predisposition variants. The disease-specific approach has the advantage of reducing the size of the assay, potentially allowing higher throughput through greater sample multiplexing. The identified variants in a focused gene set are relatively more likely to have been discussed in the literature in the context of the patient's tumor type; that is, the significance of identified variants is more likely to be known. Focused gene sets also reduce exposure to incidental variants.

Pan-cancer tests have also been implemented in several of the laboratories we surveyed, including Washington University (St Louis, Missouri)[141] and Foundation Medicine (Cambridge, Massachusetts).[142] These tests include a larger group of genes, all of which are associated with actionable variants in some tumor type. Arguments in favor of this approach include simplified development; a single assay need only be validated once, whereas multiple disease-specific assays require multiple validation procedures. The laboratory workflow is also simpler with a pan-cancer test than with multiple site-specific tests. More importantly, by sequencing genes that are not canonically mutated in the patient's cancer type, one increases the probability of discovering unexpected variants. Indeed, the promise of precision medicine is that the mutational profile of a patient's tumor may be more important than the tumor's site of origin or direction of differentiation.[143] Because the cost of testing is an important consideration, it is noteworthy that the cost of NGS-based assays does not scale directly with the number of genes tested; the costs of sequencing targeted sets of 15 or 50 genes are similar. Thus, the difference in assay size between disease-specific tests and pan-cancer tests is not accompanied by a proportionate increase in cost.

A detailed description of the strategies for validating NGS panel tests is beyond the scope of this article, but several general points are worth mentioning. First, NGS panel tests are fundamentally similar to every other laboratory test and thus require a formal analysis of factors such as their sensitivity, specificity, reproducibility, and reference range. Second, NGS panel tests involve 2 separate components: a wet-lab component (for DNA extraction, library preparation, and actual sequence generation) and an in silico component (the bioinformatic process by which variants are identified and annotated), both of which require formal validation.[144–147] Third, several regulatory agencies have promulgated requirements for validation, and it is clear that different validation approaches fulfill these requirements.[141,142,148]

Available gene panels for NGS may be either predesigned by the manufacturers of sequencing platforms or designed by individual users. Predesigned gene panels are easier and faster to validate and implement in a clinical laboratory because the manufacturer performs the initial troubleshooting and library optimization, provides step-by-step instructions for the sequencing process, and frequently supplies the analytic pipeline for data interpretation. However, the library design, gene selection, and bioinformatics pipeline are "locked" by the manufacturer. By contrast, custom gene panels allow flexibility in choosing an optimal methodologic approach (eg, hybrid capture versus amplification-based sequencing) and in selecting an appropriate sequencing platform. Custom panels allow the inclusion of a variable number of genes (targets) of interest according to the goals of the panel, and they allow the user to modify the panel as new targets become available. The development and validation of a custom panel can be tedious and time consuming. This process begins with reviews of the PubMed (US National Library of Medicine, Bethesda, Maryland) literature and various mutation databases to identify the genes, exons, or hotspots of interest. The genomic coordinates for these alterations must be submitted to a manufacturer for the synthesis of primers for a target amplification-based library or for hybrid capture probes. Next, extensive troubleshooting of the panel's accuracy and performance characteristics (such as depth and uniformity of coverage and reads on target) is performed; suboptimal results might require the redesign of the entire panel. Finally, more extensive validation is required for custom gene panels, including validation of the "wet" sequencing part and the custom bioinformatics pipeline.

The greatest challenge associated with performing NGS tests is the clinical interpretation of the results. For most tested genes, there is a finite and relatively small number of variants with well-established clinical significance (eg, the *BRAF* V600E mutation in melanoma and the *EGFR* L858R mutation in lung adenocarcinoma). When performing NGS of cancer samples, particularly when a matched constitutional sample is not available, these few, well-established mutations represent a small percentage of the alterations detected by the analysis. Most of the discovered mutations are of unclear significance, presenting the reporting laboratory with a conundrum: how much effort should be invested in analyzing the importance of each of these mutations?

Numerous informatics tools are available to assist with these interpretations: published literature searches, databases of mutations reported previously in cancer or in constitutional samples from healthy volunteers, in silico functional prediction tools that analyze the likely functional and structural consequences of the amino acid changes encoded by the mutation, and assessments of the conservation of a given nucleotide through evolution. Ultimately, however, none of these tools are sufficient for a definitive assessment. The laboratory quality health care provider issuing the report must synthesize this information and render a personal, professional opinion. An automated knowledge base can store these interpretations so that the next time a gene mutation–disease pair is encountered, time is saved; nonetheless, the effort required for the initial interpretations is significant.

An alternative is to interpret only the mutations that have a well-established meaning and to present the remainder as a list of variants of uncertain significance. This approach places the onus for interpreting these results on the practitioner who receives the report, who may not be well equipped to perform this function within the time constraints of a clinical practice. If the practitioner cannot interpret the variants of uncertain significance or explain their meaning to patients, some patients will undoubtedly attempt to interpret them themselves, relying heavily upon Web-based resources.

One potential solution would be a shared knowledge base, similar to the existing databases that catalog the presence of mutations in various genes in various tumors. In this instance, however, the functional relevance of these mutations would be included. Currently, each institution performing NGS is compiling its own private knowledge base, leading to parallelized efforts. A coordinated, cooperative effort would be more efficient in the long run. This problem will only be magnified exponentially when technology advances from panels of a few hundred genes to the entire exome and genome.

In summary, the PHC Committee of the CAP developed a cancer genomics resource list to assist the laboratory pathology and clinical oncology communities. The CGRL provides a table listing the genes offered by 19 laboratories and vendors, accompanied by annotations. The entire list is available as Cancer Genomics Resource List 2014 as supplemental digital content. A subset of the genes associated with breast cancer and their annotations are included as Table 1.

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