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# Managing the genomic revolution in cancer diagnostics

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**Abstract** Molecular tumor profiling is now a routine part of patient care, revealing targetable genomic alterations and molecularly distinct tumor subtypes with therapeutic and prognostic implications. The widespread adoption of next-generation sequencing technologies has greatly facilitated clinical implementation of genomic data and opened the door for high-throughput multigene-targeted sequencing. Herein, we discuss the variability of cancer genetic profiling currently offered by clinical laboratories, the challenges of applying rapidly evolving medical knowledge to individual patients, and the need for more standardized population-based molecular profiling.

**Keywords** NGS · Next generation sequencing · Cancer panels · Mutations

Since the sequencing of the first reference human genome by the Human Genome Project [4, 5, 21], our ability to make connections between genomics and biology has grown exponentially. The field of medical oncology has seen a dramatic change in cancer therapy from the use of relatively nonselective cytotoxic agents to rationally designed therapies targeting specific molecular alterations (e.g., tyrosine kinase inhibitors in lung cancers with activating mutations in the epidermal growth factor receptor (*EGFR*) genes). Table 1 contains a partial list of current Food and Drug Administration (FDA) approved targeted cancer therapies for solid and hematologic malignancies with their molecular targets and corresponding companion diagnostics, if applicable. Some of these agents are classified as new molecular entities, which contain active moieties that have not been previously approved by the FDA, while the majority fall under new indications for existing drugs.

Notably, agents that target highly specific molecular abnormalities (e.g., the breakpoint cluster region (*BCR*)-Abelson murine leukemia viral oncogene homolog (*ABL*) gene fusion) by definition restrict the patient population that can benefit from a specific approach. In contrast, successfully modulating less specific targets (either surface receptors or targets common to many biologic processes) will likely affect a large number of patients suffering from cancer and other disorders. Checkpoint inhibitors, for example, target the programmed cell death pathway (i.e., PD-1/PD-L1). By blocking this pathway, these drugs help reactivate the body's own immune system to fight cancer cells. In a landmark decision, the FDA recently accelerated the approval of pembrolizumab for the treatment of adult and pediatric patients with microsatellite instability-high or mismatch repair deficient solid tumors that have progressed after prior treatment and who have no satisfactory alternative treatment options [10]. As the first tissue/site-agnostic genetically matched indication of its kind, pembrolizumab's approval by the FDA signifies the remarkable progress that has been made with the use of immunotherapy and the specific events that enable malignant disease.

Although biomarker discovery is thriving, incorporation of multi-analyte molecular assays into clinical practice continues to lag behind. Some of the challenges to clinical adoption include assay variability, inadequate reporting, and practical

The original version of this article was revised: Entries were incorrectly aligned in Table 2.

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**Table 1** FDA-approved targeted cancer therapies and associated companion diagnostics

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| Abiraterone acetate (Zytiga®) | Prodrug, CYP17A1 inhibitor | 12/10/2012, 4/28/2011 | Castration-resistant prostate cancer | |
| Ado-trastuzumab emtansine (Kadcyla®) | HER2/neu receptor antagonist with conjugated cytotoxin | 2/22/2013 | HER2-positive breast cancer | Dako Denmark A/S IHC HercepTest; Dako Denmark HER2 FISH PharmDx Kit |
| Afatinib dimaleate (Gilotrif®) | Irreversible covalent inhibitor of ErbB family including EGFR and HER2 with activity against *EGFR* T790 M mutations | 7/12/2014 | Non-small cell lung cancer with *EGFR* exon 19 deletions or exon 21 L858R mutations | Qiagen Manchester, Ltd. Therascreen® *EGFR* RGQ PCR Kit |
| Alectinib (Alecensa®) | Dual tyrosine kinase inhibitor of ALK and RET | 12/11/2015 | ALK-positive non-small cell lung cancer | |
| Alemtuzumab (Campath) | Anti-CD52 monoclonal antibody | 9/19/2007, 5/2001 | B cell chronic lymphocytic leukemia | |
| Aliretinoin (Panretin®) | Retinoic acid receptor agonist | 3/2/1999 | Kaposi's sarcoma | |
| Anastrozole (Arimidex®) | Reversible aromatase inhibitor | 9/1/2000, 1/1996 | Hormone receptor-positive or unknown breast cancer | |
| Atezolizumab (Tecentriq®) | PD-L1 inhibitor (monoclonal antibody) | 10/18/2016 | Non-small cell lung cancer | |
| | | 5/18/2016 | Urothelial carcinoma | |
| Avelumab (Bavencio®) | PD-L1 inhibitor (monoclonal antibody) | 5/9/2017 | Urothelial carcinoma | |
| | | 3/23/2017 | Merkel cell carcinoma | |
| Axitinib (Inlyta®) | Multi-kinase inhibitor (VEGFR1/2/3, c-kit, PDGFR) | 1/27/2012 | Renal cell carcinoma | |
| Belinostat (Beleodaq®) | Histone deacetylase inhibitor | 7/3/2014 | Peripheral T cell lymphoma | |
| Bevacizumab (Avastin®) | VEGF-A inhibitor (monoclonal antibody) | 11/14/2014 | Epithelial ovarian, fallopian tube, or primary peritoneal cancer | |
| | | 8/14/2014 | Cervical cancer | |
| | | 1/23/2013, 6/20/2006, 2/26/2004 | Colorectal cancer | |
| | | 7/31/2009 | Renal cell carcinoma | |
| | | 5/5/2009 | Glioblastoma | |
| | | 10/11/2006 | Non-squamous non-small cell lung cancer | |
| Blinatumomab (Blincyto®) | Bispecific CD19-directed CD3 T cell engager | 10/3/2014 | B cell precursor acute lymphoblastic leukemia (Philadelphia chromosome negative) | |
| Bortezomib (Velcade®) | Proteasome inhibitor | 6/23/2008 | Multiple myeloma | |
| | | 12/8/2006 | Mantle cell lymphoma | |
| Bosutinib (Bosulif) | Bcr-Abl tyrosine kinase competitive inhibitor | 9/4/2012 | Chronic myelogenous leukemia (Philadelphia chromosome positive) | |
| Brentuximab vedotin (Adcetris®) | Anti-CD30 monoclonal antibody | 8/17/2015 | Hodgkin lymphoma | |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| | | 8/19/2011 | Anaplastic large cell lymphoma | |
| Brigatinib (Alunbrig™) | Dual tyrosine kinase inhibitor of ALK and EGFR | 4/28/2017 | ALK-positive non-small cell lung cancer | |
| Cabazitaxel (Jevtana®) | Microtubule inhibitor | 6/17/2010 | Hormone-refractory prostate cancer | |
| Cabozantinib-S-malate (Cabometyx®, Cometriq®) | Multi-kinase inhibitor (RET, c-MET, VEGFR1/2/3, AXL, ROS1, TYRO3, MER, c-kit, TRKB, FLT3, TIE2) | 4/25/2016 | Renal cell carcinoma | |
| | | 11/29/2012 | Medullary thyroid cancer | |
| Carfilzomib (Kyprolis®) | Selective proteasome inhibitor | 7/24/2015, 7/20/2012 | Multiple myeloma | |
| Ceritinib (Zykadia®) | ALK inhibitor | 4/29/2014 | ALK-positive non-small cell lung cancer | |
| Cetuximab (Erbitux®) | EGFR antagonist (monoclonal antibody) | 7/6/2012, 10/2/2007, 2/12/2004 | KRAS wild-type, EGFR-expressing colorectal cancer | Roche Cobas® *KRAS* Mutation test, Qiagen Manchester, Ltd. Therascreen® *KRAS* RGQ PCR kit; Dako North America, Inc. *EGFR* PharmDx Kit (IHC) |
| | | 11/7/2011, 3/1/2006 | Squamous cell carcinoma of the head and neck | |
| Cobimetinib (Cotellic®) | Reversible inhibitor of MEK1/2 | 11/10/2015 | Melanoma with $BRAF^{V600E}$ or $^{V600K}$ mutation | |
| Crizotinib (Xalkori®) | Multi-kinase inhibitor (ALK, HGFR, c-Met, ROS1, RON) | 3/11/2016 | ROS-1-positive non-small cell lung cancer | |
| | | 11/20/2013, 8/26/2011 | ALK-positive non-small cell lung cancer | Ventana Medical Systems, Inc. ALK (D5F3) CDx IHC Assay; Abbott Molecular, Inc. Vysis *ALK* Break Apart FISH Probe Kit |
| Dabrafenib (Tafinlar®) | Inhibitor of some mutated forms of BRAF, as well as wild-type BRAF and CRAF | 1/10/2014, 5/29/2013 | Melanoma with $BRAF^{V600E}$ or $^{V600K}$ mutation | bioMérieux, Inc. THxID™ BRAF Kit |
| Daratumumab (Darzalex™ injection) | Anti-CD38 monoclonal antibody | 11/21/2016, 11/16/2015 | Multiple myeloma | |
| Dasatinib (Sprycel) | Bcr-Abl tyrosine kinase inhibitor | 10/28/2010, 6/28/2006 | Chronic myelogenous leukemia (Philadelphia chromosome positive) | |
| | | 6/28/2006 | Acute lymphoblastic leukemia (Philadelphia chromosome positive) | |
| Denosumab (Xgeva®) | RANKL inhibitor (monoclonal antibody) | 6/13/2013 | Giant cell tumor of bone | |
| Dinutuximab (Unituxin®) | Anti-GD2 monoclonal antibody | 3/10/2015 | High-risk neuroblastoma | |
| Durvalumab (Imfinzi®) | PD-L1 inhibitor (monoclonal antibody) | 5/1/2017 | Urothelial carcinoma | Ventana Medical Systems, Inc. PD-L1 (SP263) assay |
| Elotuzumab (Empliciti™) | Anti-SLAMF7 monoclonal antibody | 11/30/2015 | Multiple myeloma | |
| Enzalutamide (Xtandi®) | Androgen receptor inhibitor | 8/31/2012 | Castration-resistant prostate cancer | |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| Eribulin (Halaven® injection) | Microtubule inhibitor | 1/28/2016 | Liposarcoma | |
| Erlotinib hydrochloride (Tarceva®) | Reversible EGFR inhibitor with higher affinity for *EGFR* exon 19 deletion or exon 21 L858R mutation | 10/18/2016, 5/14/2013 | Non-small cell lung cancer with *EGFR* exon 19 deletions or exon 21 L858R mutations | Roche Molecular Systems, Inc. Cobas® *EGFR* Mutation Test |
| | | 11/2/2005 | Pancreatic carcinoma | |
| Everolimus (Afinitor®) | mTOR inhibitor | 2/26/2016 | Well-differentiated non-functional, neuroendocrine tumors of gastrointestinal or lung origin | |
| | | 8/29/2012, 10/29/2010 | Subependymal giant cell astrocytoma | |
| | | 7/20/2012 | Hormone receptor-positive, HER2-negative breast cancer | |
| | | 5/5/2011 | Neuroendocrine tumors of pancreatic origin | |
| | | 3/30/2009 | Renal cell carcinoma | |
| Exemestane (Aromasin®) | Irreversible aromatase inhibitor | 10/5/2005 | Estrogen receptor-positive breast cancer | |
| Fulvestrant (Faslodex®) | Selective estrogen receptor degrader (SERD) | 4/25/2002 | Hormone receptor-positive breast cancer | |
| Gefitinib (Iressa®) | Reversible EGFR inhibitor | 7/13/2015, 6/17/2005 | Non-small cell lung cancer with EGFR exon 19 deletions or exon 21 L858R mutations | Qiagen Manchester, Ltd. Therascreen® *EGFR* RGQ PCR Kit |
| Ibritumomab tiuxetan (Zevalin®) | Anti-CD20 monoclonal antibody | 2/2002 | Non-Hodgkin's lymphoma | |
| Ibrutinib (Imbruvica®) | BTK inhibitor | 11/13/2013 | Mantle cell lymphoma | |
| | | 7/2014, 2/12/2014 | Chronic lymphocytic leukemia with 17p deletion | |
| | | 1/29/2015 | Waldenstrom's macroglobulinemia | |
| Idelalisib (Zydelig®) | PI3Kδ kinase inhibitor | 7/23/2014 | Chronic lymphocytic leukemia | |
| | | 7/23/2014 | Follicular B cell non-Hodgkin lymphoma | |
| Imatinib mesylate (Gleevac®) | Abl, c-kit, and PDGFR tyrosine kinase inhibitor | 10/19/2006 | Dermatofibrosarcoma protuberans (DFSP) | |
| | | 1/31/2012, 2/1/2001 | Kit-positive malignant gastrointestinal stromal tumors | Dako North America, Inc. C-kit PharmDx |
| | | 10/19/2006 | Aggressive systemic mastocytosis | ARUP Laboratories, Inc. *KIT* D816V Mutation Detection by PCR for Gleevac |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| | | | | Eligibility in Aggressive Systemic Mastocytosis (ASM) |
| | | 10/19/2006 | Myelodysplastic/myeloproliferative diseases | ARUP Laboratories, Inc. *PDGFRB* FISH for Gleevac Eligibility in Myelodysplastic Syndrome/Myeloproliferative Disease (MDS/MPD) |
| | | 10/19/2006 | Acute lymphocytic leukemia (Philadelphia chromosome positive), hypereosinophilic syndrome/chronic eosinophilic leukemia | |
| | | 9/27/2006, 5/2001 | Chronic myelogenous leukemia (Philadelphia chromosome positive) | |
| Ipilimumab (Yervoy®) | CTLA-4 inhibitor (monoclonal antibody) | 10/28/2015, 3/25/2011 | Melanoma | |
| Ixazomib citrate (Ninlaro®) | Proteasome inhibitor | 11/20/2015 | Multiple myeloma | |
| Lanreotide acetate (Somatuline® depot) | Somatostatin analog | 12/16/2014 | Well or moderately differentiated gastroenteropancreatic neuroendocrine tumors | |
| Lapatinib ditosylate (Tykerb®) | Dual tyrosine kinase inhibitor of ErbB-2 and ErbB-1 | 1/29/2010, 3/13/2007 | Hormone receptor-positive, HER2-positive breast cancer | |
| Lenvatinib mesylate (Lenvima®) | Multi-kinase inhibitor (VEGFR1/2/3, FGFR1/2/3/4, PDGFR-alpha, c-kit, RET) | 5/13/2016 | Renal cell carcinoma | |
| | | 2/13/2015 | Differentiated thyroid cancer | |
| Letrozole (Femara®) | Reversible aromatase inhibitor | 12/28/2005, 10/29/2004 | Hormone receptor-positive breast cancer | |
| Midostaurin (Rydapt®) | FLT3 inhibitor | 4/28/2017 | *FLT3*-mutated acute myeloid leukemia | Invivoscribe Technologies, Inc. LeukoStrat® CDx *FLT3* Mutation Assay |
| | | 4/28/2017 | Aggressive systemic mastocytosis, mast cell leukemia | |
| Necitumumab (Portrazza®) | EGFR inhibitor (monoclonal antibody) | 11/24/2015 | Squamous non-small cell lung cancer | |
| Nilotinib (Tasigna®) | Selective Bcr-Abl tyrosine kinase inhibitor | 6/17/2010, 10/29/2007 | Chronic myelogenous leukemia (Philadelphia chromosome positive) | |
| Niraparib (Zejula®) | PARP inhibitor | 3/27/2017 | Epithelial ovarian, fallopian tube, or primary peritoneal cancer | |
| Nivolumab (Opdivo® injection) | PD-1 inhibitor (monoclonal antibody) | 2/2/2017 | Urothelial carcinoma | |
| | | 11/10/2016 | | |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| | | | Squamous cell carcinoma of the head and neck | |
| | | 11/23/2015 | Renal cell carcinoma | |
| | | 10/9/2015 | Non-small cell lung cancer | |
| | | 9/30/2015, 12/22/2014 | *BRAF* V600 wild-type melanoma | |
| | | 5/17/2016 | Hodgkin lymphoma | |
| | | 3/4/2015 | Squamous non-small cell lung cancer | |
| Obinutuzumab (Gazyva®) | Anti-CD20 monoclonal antibody | 11/1/2013 | Chronic lymphocytic leukemia | |
| | | 2/26/2016 | Follicular lymphoma | |
| Ofatumumab (Arzerra®, HuMax-CD20®) | Anti-CD20 monoclonal antibody | 1/19/2016, 4/17/2014, 10/26/09 | Chronic lymphocytic leukemia | |
| Olaparib (Lynparza®) | PARP inhibitor | 12/19/2014 | Ovarian cancer associated with deleterious *BRCA1/2* mutations | Myriad Genetic Laboratories, Inc. BRACAnalysis CDx™ |
| Olaratumab (Lartruvo®) | PDGFR-alpha inhibitor (monoclonal antibody) | 10/19/2016 | Soft tissue sarcoma | |
| Osimertinib (Tagrisso™) | Irreversible EGFR inhibitor with activity against activating, sensitizing *EGFR* mutations and T790 M mutation | 3/30/2017, 11/13/2015 | Non-small cell lung cancer with *EGFR* T790 M mutation | Roche Molecular Systems, Inc. Cobas® *EGFR* Mutation Test v2 |
| Palbociclib (Ibrance®) | Selective inhibitor of CDK4 and CDK6 | 3/31/2017, 2/19/2016, 2/3/2015 | Hormone receptor-positive, HER2-negative breast cancer | |
| Panitumumab (Vectibix®) | EGFR antagonist (monoclonal antibody) | 9/27/2006 | EGFR-expressing colorectal cancer | Roche Molecular Systems, Inc. Cobas® *KRAS* Mutation test; Qiagen Manchester, Ltd. Therascreen® *KRAS* RGQ PCR kit; Dako North America, Inc. *EGFR* PharmDx Kit |
| Panobinostat (Farydak®) | Histone deacetylase inhibitor | 2/23/2015 | Multiple myeloma | |
| Pazopanib hydrochloride (Votrient®) | Multi-kinase inhibitor (VEGFR, PDGFR, FGFR, c-kit) | 4/26/2012 | Soft tissue sarcoma | |
| | | 10/19/2009 | Renal cell carcinoma | |
| Pembrolizumab (Keytruda®) | PD-L1 inhibitor (monoclonal antibody) | 5/23/2017 | MSI-H or mismatch repair deficient solid tumors | |
| | | 5/23/2017 | MSI-H or mismatch repair deficient colorectal cancer | |
| | | 5/18/2017 | Urothelial carcinoma | |
| | | 5/10/2017 | Non-squamous non-small cell lung cancer | |
| | | 10/24/2016, 10/2/2015 | Non-small cell lung cancer | Dako North America, Inc. PD-L1 IHC 22C3 PharmDx |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| | | 3/15/2017 | Classical Hodgkin lymphoma | |
| | | 8/5/2016 | Squamous cell carcinoma of the head and neck | |
| | | 12/18/2015, 9/4/2014 | Melanoma | |
| Pertuzumab (Perjeta®) | HER2/neu receptor antagonist (monoclonal antibody) | 9/30/2013, 6/8/2012 | HER2-positive breast cancer | Dako North America, Inc. IHC HercepTest, Dako HER2 FISH PharmDx Kit |
| Ramucirumab (Cyramza®) | VEGFR-2 antagonist (monoclonal antibody) | 4/24/2015 | Colorectal cancer | |
| | | 12/12/2014 | Non-small cell lung cancer | |
| | | 4/21/2014 | Gastric or gastroesophageal junction adenocarcinoma | |
| Regorafenib (Stivarga®) | Multi-kinase inhibitor targeting angiogenic, stromal, and oncogenic receptor tyrosine kinases including VEGFR2 and TIE2 | 4/27/2017 | Hepatocellular carcinoma | |
| | | 2/25/2013 | Gastrointestinal stromal tumors | |
| | | 9/27/2012 | Colorectal cancer | |
| Ribociclib (Kisqali®) | CDK4/6 inhibitor | 3/13/2017 | Hormone receptor-positive, HER2-negative breast cancer | |
| Rituximab (Rituxan®, Mabthera®) | Anti-CD20 monoclonal antibody | 10/19/2012, 1/28/2011, 9/29/2006, 2/10/2006 | CD20-positive B cell non-Hodgkin's lymphoma | |
| | | 2/18/2010 | Chronic lymphocytic leukemia | |
| Romidepsin (Istodax®) | Histone deacetylase inhibitor | 11/6/2009 | Cutaneous and peripheral T cell lymphoma | |
| Rucaparib camsylate (Rubraca®) | PARP inhibitor | 12/19/2016 | Ovarian cancer associated with deleterious *BRCA1/2* mutations | Foundation Medicine FoundationFocus™ CDx*BRCA* Assay |
| Ruxolitinib (Jakafi®) | JAK1/2 inhibitor | 10/4/2014, 11/16/2011 | Polycythemia vera, primary myelofibrosis | |
| Siltuximab (Sylvant®) | Anti-IL-6 monoclonal antibody | 4/23/2014 | Multicentric Castleman's disease (HIV-negative, HHV8-negative) | |
| Sonidegib (Odomzo®) | Hedgehog pathway inhibitor | 7/24/2015 | Basal cell carcinoma | |
| Sorafenib tosylate (Nexavar®) | Multi-kinase inhibitor (VEGFR2/3, PDGFR-beta, c-kit, and Raf family kinases including BRAF) | 11/22/2013 | Differentiated thyroid carcinoma | |
| | | 11/16/2007 | Hepatocellular carcinoma | |
| | | 10/20/2005 | Renal cell carcinoma | |
| Sunitinib malate (Sutent®) | Multi-kinase inhibitor (PDGFR-alpha, PDGFR-bea, VEGFR1/2/3, c-kit, FLT3, CSF-1R, and RET) | 5/20/2011 | Well-differentiated pancreatic neuroendocrine tumors | |
| | | 1/26/2006 | Renal cell carcinoma | |
| | | 1/26/2006 | Gastrointestinal stromal tumors | |

**Table 1** (continued)

| FDA-approved drug | Pharmacodynamics/cellular target(s) | Drug approval date | Tumor type | FDA-approved companion diagnostic test |
|---|---|---|---|---|
| Tamoxifen citrate (Nolvadex®) | Prodrug, active metabolites act as estrogen receptor antagonists | 1977 | Estrogen receptor-positive or unknown breast cancer | |
| Temsirolimus (Torisel®) | mTOR inhibitor | 5/30/2007 | Renal cell carcinoma | |
| Toremifene citrate (Fareston®) | Selective estrogen receptor modulator (SERM) | 1997 | Estrogen receptor-positive or unknown breast cancer | |
| Trametinib (Mekinist®) | Reversible inhibitor of MEK1/2 | 1/10/2014, 5/29/2013 | Melanoma with *BRAF*$^{V600E}$ or $^{V600K}$ mutation | bioMérieux, Inc. THxID™ BRAF Kit |
| Trastuzumab (Herceptin®) | HER2/neu receptor antagonist (monoclonal antibody) | 10/20/2010 | Gastric or gastroesophageal junction adenocarcinoma | Dako Denmark A/S HercepTest, Dako Denmark HER2 FISH PharmDx Kit |
| | | 11/16/2006, 9/1998 | Breast cancer | Ventana Medical Systems, Inc. Inform HER2/neu FISH; Abbott Molecular, Inc. PathVysion HER-2 FISH; Ventana PATHWAY anti-HER-2/neu monoclonal antibody; Biogenex Laboratories InSite HER2/neu monoclonal antibody; Ventana Inform HER2 Dual ISH DNA Probe Cocktail; Life Technologies SPOT-Light HER2 CISH Kit; Dako Denmark A/S HER2 CISH PharmDx Kit; Dako Denmark A/S HercepTest; Dako Denmark A/S HER2 FISH PharmDx Kit; Leica Biosystems Bond Oracle HER2 IHC system |
| Vandetanib (Caprelsa®) | Multi-kinase inhibitor (VEGFR, EGFR, RET, BRK, TIE2, and members of the EPH receptor and Src kinase families) | 4/6/2011 | Medullary thyroid cancer | |
| Vemurafenib (Zelboraf®) | Inhibitor of some mutated forms of *BRAF* | 8/17/2011 | Melanoma with BRAF$^{V600E}$ mutation | Roche Molecular Systems, Inc. Cobas® 4800 *BRAF* V600 Mutation Test |
| Venetoclax (Venclexta®) | Bcl-2 inhibitor | 4/11/2016 | Chronic lymphocytic leukemia with 17p deletion | Abbott Molecular, Inc. Vysis CLL FISH Probe Kit |
| Vismodegib (Erivedge®) | Hedgehog pathway inhibitor | 1/30/2012 | Basal cell carcinoma | |
| Vorinostat (Zolinza®) | Histone deacetylase inhibitor | 10/6/2006 | Cutaneous T cell lymphoma | |
| Ziv-aflibercept (Zaltrap®) | VEGF-A, VEGF-B, and PGF inhibitor | 8/3/2012 | Colorectal cancer | |

The above information was compiled from www.cancer.gov, www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm, and www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm (last accessed on May 25, 2017). It is not a comprehensive list

*IHC* immunohistochemistry

obstacles such as the following: lack of resources, personnel, or expertise in smaller clinical laboratories [11]. In addition, knowledge of the specific tumor markers that have the greatest value in care evolve over time, leading to inconsistencies in analysis and interpretation.

Significant efforts have been made to standardize the development and reporting of diagnostic, prognostic, and predictive tumor markers [23] since the FDA started requiring co-development of targeted therapies with companion diagnostic tests. Regulatory oversight of assay development, however, is relatively limited, especially regarding the steps required to incorporate tumor markers into clinical care and to expand testing for therapeutic drug classes rather than a single agent. An important exception to the requirement for co-development of companion diagnostics is for promising therapeutics intended to treat tumors for which no satisfactory alternative treatment exists and the benefits outweigh the risks of using a non-FDA approved diagnostic test. Most companion diagnostics are relatively simple, analyzing 1 to 2 genetic biomarkers or are based on multivariate index assays that derive a score, probability, or classification (e.g., Oncotype DX). As a result, most medium to large clinical labs design their own laboratory developed tests (LDTs) to more rapidly translate new scientific knowledge into medical practice. These tests detect genetic variations relevant to approved targeted therapies, as well as emerging biomarkers actively under investigation in clinical trials or other research studies.

Not surprisingly, there is considerable variation between laboratories in the type of LDTs that they offer. Both academic institutions and commercial reference laboratories have developed customized gene panels for targeted sequencing. These panels, however, are not static and are continually updated as new knowledge become available (Tables 2 and 3). Some panels are organ or system-specific, whereas others are pan-cancer tests. The largest pan-cancer panels typically involve 200 to 400 genes. Many academic institutions validate ready-made vendor kit solutions, such as the Ion Torrent AmpliSeq™ Cancer Hotspot Panel (Thermo Fisher Scientific, Waltham, MA), Oncomine™ Comprehensive Assay (Thermo Fisher Scientific), or Illumina TruSeq™ and TruSight™ sequencing panels (Illumina, San Diego, CA), while others use custom-designed panels with genes of their choosing. The overlap between genes covered by each panel is variable, which reflects the general lack of standardization of molecular testing and difficulty of defining a clinically relevant cancer gene set.

Although some tumors depend almost exclusively on the oncogenic activity of a protein mutation in its early stages of development, most malignant conditions accumulate multiple genetic alterations within subclonal populations that ultimately dictate tumor growth. This genetic heterogeneity of tumors along with minimal tissue requirements for testing and financial and time constraints makes it challenging to perform accurate and comprehensive genomic testing on tumor samples. In addition, rapid advances in testing technology and understanding of tumor biology can quickly render a testing method or target obsolete.

Unfortunately, there is little consensus on how physicians should use multiplex gene testing for personalized cancer care beyond the genes in the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (NCCN Guidelines) [7, 11, 27]. For example, testing for *EGFR*, *KRAS*, *ALK*, and *ROS1* are the only genes clearly described in the Principles of Pathologic Review in the latest version (6.2017) of the NCCN Guidelines for non-small cell lung cancer [9]. Although targeted agents for emerging biomarkers, including *HER2* mutations, *BRAF* V600E mutation, *RET* rearrangements, and *MET* amplification or exon 14 skipping mutation, are acknowledged, they have not been incorporated into the decision trees. Instead, the NCCN "strongly advises broader molecular profiling" and encourages enrollment of any patient with cancer in a clinical trial. The scope and type of molecular profiling, however, is not explained in detail [8, 9].

Nevertheless, great interest remains from all stakeholders in cancer care, including patients, to translate the latest research findings into clinical care. The desire for early adoption before assessment of clinical utility is performed, has led many to question the true value of molecular testing beyond what is deemed actionable at the time testing is performed, especially if multiplex testing includes both clinically useful markers and markers with undetermined clinical utility or significance. However, variants identified through multiplex testing are being used to direct patients to appropriate trials and researchers of molecular events that may influence a particular patient's response to treatment.

Although multiplex testing is frequently used in practice, as the number of genes implicated in cancer genetics, prognosis, and treatment increase, some are considering shifting to whole exome sequencing (WES). WES has the obvious advantage of probing a larger portion of the protein-coding genome, including all cancer-related genes present in the smaller targeted gene panels. This technique is becoming increasingly facile and cost-effective, and some groups have even demonstrated its feasibility in the clinical environment. What does this mean for the next generation of clinical oncology molecular tests? Is bigger always better? Instead of continually expanding targeted gene panels, should labs switch to whole exome (22,000 protein-coding genes) or whole genome (including non-coding regions) sequencing?

In the research setting, WES has led to the identification of novel gene fusions, cryptic splice sites, and genes not previously implicated in the tumor being tested. The large volume of data generated and the computational and analytical challenges associated with the interpretation of results have limited its widespread clinical implementation. Whole genome

**Table 2** Select academic laboratories offering solid tumor panels with >20 genes (compiled as of March 6, 2017)

| Clinical laboratory | Test name | Panel details | Methodology/ platform | Tumor/ normal sequencing | Turnaround time (TAT) | CPT® Code(s) | URL source/Reference |
|---|---|---|---|---|---|---|---|
| Brigham and Women's Hospital, Center for Advanced Molecular Diagnostics Boston, MA | OncoPanel | 275 genes (full coding regions) plus selected intronic regions of 30 genes | Hybrid-capture, Illumina (HiSeq™) | Plans to implement in the future | 3 weeks | Not specified | [12] |
| City of Hope Clinical Molecular Diagnostic Laboratory Duarte, CA | OncoMutations Panel | 49 genes (mutation hotspots) | Amplicon-based, Ion AmpliSeq™ technology | No | 14 days | 81445, 88381, G0452 | https://www.cityofhope.org/clinical-molecular-diagnostic-laboratory/list-of-cmdl-tests/oncomutations |
| | OncoComplete (OncoMutations PLUS OncoFusions) | 49 genes (mutation hotspots), fusion transcripts for 53 genes | Amplicon-based, Ion AmpliSeq™ technology | No | 14 days | 81445, 81479, 88381, G0452(×2) | https://www.cityofhope.org/clinical-molecular-diagnostic-laboratory/list-of-cmdl-tests/oncomutations |
| Columbia University Laboratory for Personalized Genomic Medicine New York, NY | TruSeq Targeted Cancer Panel | 48 genes | Amplicon-based, Illumina (TruSeq®) | No | 30 days | 81445, 88381 | http://pathology.columbia.edu/diagnostic/PGM/oncologytests.html |
| | Combined Cancer Panel (CCCP) | 467 genes (393-exons only; 74-whole genes) | Amplicon-based, Illumina (TruSeq®) | No | 30 days | 81455, 88381 | http://pathology.columbia.edu/diagnostic/PGM/oncologytests.html |
| Duke University Health System Durham, NC | Solid Tumor Hotspot NGS Panel | 50 genes (mutation hotspots) | Amplicon-based, Ion AmpliSeq™ technology | No | 14 days | 81445 | http://dukemolecular.duhs.duke.edu/RequestForms/Duke_CHPv2_information.pdf |
| Emory University Molecular Diagnostics Lab Atlanta, GA | Cancer Mutation Panel 26 (CMP26) | 26 genes (whole-exon coverage) | Amplicon-based, Illumina (TruSight® Tumor 26) | No | 7–10 days | 81445, G0452 | https://www.testmenu.com/emory/Tests/352670 |
| Jefferson Cancer Genomics Laboratory Philadelphia, PA | Ion Ampliseq™ Cancer Panel | 50 genes | Amplicon-based, Ion AmpliSeq™ technology | No | 14 days | Not specified | http://www.jefferson.edu/university/jmc/departments/cancer-biology/research/cancer-genomics/services.html |
| Johns Hopkins Molecular Diagnostics Laboratory Baltimore, MD | 50 Gene Panel | 50 genes (mutation hotpots) | Amplicon-based, Ion AmpliSeq™ technology | No | 10–14 days | 81445 | http://pathology.jhu.edu/MolecularDiagnostics/tests.cfm |
| Massachusetts General Hospital, Center for Integrated Diagnostics Boston, MA | NGS SNaPshot | 39 genes (mutation hotspots) | Primer single-base extension, SNaPShot™ methodology | No | 3 weeks | Not specified | http://www.massgeneral.org/pathology/research/resourcelab.aspx?id=74 |
| Memorial Sloan Kettering Cancer Center New York, NY | Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™) | 410 genes, 17 rearrangements | Hybrid-capture, Illumina (HiSeq™) | Yes | Not specified | Not specified | [34] |
| New York Presbyterian Hospital/Weill Cornell Molecular Pathology Laboratory New York, NY | Cancer Gene Mutation Panel-50 (CGMP-50) | 50 genes (mutation hotspots) | Amplicon-based, Ion AmpliSeq™ technology | No | 7–10 days | 81445 | http://cornellpathology.com/clinical-services/molecular-and-genomic-pathology |
| Oregon Health & Science University, Knight Diagnostic Laboratories Portland, OR | GeneTrails® Comprehensive Solid Tumor Panel | 124 genes | Amplicon-based, Illumina (NextSeq™) | No | 14 days | 81455 | https://www.ohsu.edu/custom/knight-diagnostic-labs/featured/introducing/genetrails-solid-tumor-panel |
| | GeneTrails® Solid Tumor Fusion Gene Panel | fusions in 20 target genes, gene expression for 8 genes | Next generation RNA sequencing, confirmation by FISH | No | 14–17 days | 81455 | https://www.ohsu.edu/custom/knight-diagnostic-labs/home/test-details?id=GeneTrails+Solid+Tumor+Fusion+Gene+Panel |
| Stanford Molecular Pathology Laboratory Palo Alto, CA | Solid Tumor Actionable Mutation Panel (STAMP) | 130 genes (either in part or fully) | Hybrid-capture, Illumina (MiSeq™) | No | 28 days | 81455 | http://www.stanfordlab.com/esoteric/test-stanford-solid-tumor-actionable-mutation-panel.html |
| | Cancer Somatic Mutation Panel, Non-Blood | 48 genes (targeted regions and hotspot mutations) | Hybrid-capture, Illumina (MiSeq™); primer single-base extension, SNaPShot™ methodology for 2 genes | No | 28 days | 81445 | http://stanfordlab.com/esoteric/test-cancer-somatic-mutation-panel-non-blood.html |

**Table 2** (continued)

| Clinical laboratory | Test name | Panel details | Methodology/ platform | Tumor/ normal sequencing | Turnaround time (TAT) | CPT® Code(s) | URL source/Reference |
|---|---|---|---|---|---|---|---|
| The Ohio State University Wexner Medical Center Molecular Pathology Laboratory Columbus, OH | Lung Cancer Mutation Panel (PULMOL) | 26 genes (commonly mutated regions) | Amplicon-based, Ion AmpliSeq™ technology | No | Not specified | 81445, G0452 | http://pathology.osu.edu/ext/divisions/Clinical/molpath/tests.html |
| | Colon Cancer Mutation Panel (COLMOL) | 26 genes (commonly mutated regions) | Amplicon-based, Ion AmpliSeq™ technology | No | Not specified | 81445, G0452 | http://pathology.osu.edu/ext/divisions/Clinical/molpath/tests.html |
| UC San Diego Health, Center for Advanced Laboratory Medicine, Clinical Genomics Laboratory San Diego, CA | Comprehensive NGS Solid Tumor Mutation Panel | 397 genes (all exons), transcripts for 28 genes | NGS | Yes | 16 days | Not specified | https://www.testmenu.com/ucsd/Tests/565799 |
| UC San Francisco Clinical Cancer Genomics Laboratory San Francisco, CA | UCSF500 Cancer Gene Panel | ~500 genes (including select introns), fusions involving 42 genes | Hybrid-capture, Illumina (HiSeq™) | Yes | 3–4 weeks | Not specified | http://cancer.ucsf.edu/intranet/ccgl |
| University of Chicago Molecular Pathology Laboratory Chicago, IL | OncoPlus Universal Cancer Mutation Analysis Panel | 147 clinically reported genes, fusions in *ALK, RET, ROS1* | Hybrid-capture, Illumina (HiSeq™) | No | 14–20 days | 81455 | http://uchicagomedlabs.testcatalog.org/show/NGPLSF |
| University of Nebraska Medical Center Regional Pathology Services Omaha, NE | 50 Gene Panel | 50 genes (mutation hotpots) | Amplicon-based, Ion AmpliSeq™ technology | No | 10–14 days | Not specified | https://www.testmenu.com/nebraska/Tests/533515 |
| University of Pennsylvania, Center for Personalized Diagnostics Philadelphia, PA | Comprehensive Solid Tumor Panel | 153 genes (mutation hotspots) | Amplicon-based, Agilent HaloplexHS enrichment, Illumina (MiSeq™) | No | ≤ 21 days | Not specified | http://pathology.med.upenn.edu/clinical-services/center-for-personalized-diagnostics |
| University of Pittsburgh Medical Center Molecular & Genomic Pathology Laboratory Pittsburgh, PA | OncoSeq | 28 genes (mutation hotspots) | Amplicon-based, Ion AmpliSeq™ technology | No | 7 days | Not specified | http://mgp.upmc.com/Applications/mgp/Home/Test/OncoSeq_Details |
| | Personalized Cancer Mutation Panel | 50 genes (targeted regions) | Amplicon-based, Ion AmpliSeq™ technology | Yes, for LOH and MSI analyses | 7–14 days | Not specified | http://mgp.upmc.com/Applications/mgp/Home/Test/PCMP_Details |
| University of Texas MD Anderson Cancer Center Houston, TX, USA | Ion Torrent AmpliSeq™ Cancer Panel | 46 genes (mutation hotspots) | Amplicon-based, Ion AmpliSeq™ technology | No | Not specified | Not specified | [32] |
| University of Texas Southwestern Medical Center, Veripath Laboratory Dallas, TX | Cancer Mutation 50-Gene Panel | 50 genes | Amplicon-based, Ion Torrent PGM™ | No | < 20 days | 81455, 88381 | http://veripath.swmed.edu/LabDetails.aspx?TitleID=TEMP618201594638 |
| University of Washington Medical Center Genetics Laboratory Seattle, WA | UW-OncoPlex™ | 262 genes (select regions and introns) | Hybrid-capture, Illumina (HiSeq™, MiSeq™) | No | 4–6 weeks | 81455 (88381 if applicable) | http://web.labmed.washington.edu/tests/genetics/UW-OncoPlex |
| Washington University Genomics and Pathology Services St. Louis, MO | Solid Tumor Gene Set | 65 genes (all coding region and select introns) | Hybrid-capture, Illumina (HiSeq™) | No | 3 weeks | 81455 | https://gps.wustl.edu/patient-care/sequencing-tests/ |

**Table 3** Select reference laboratories offering solid tumor panels with >20 genes (compiled as of March 6, 2017)

| Company/ laboratory | Test name | DNA/ RNA | Panel details | Methodology | Tumor/ normal sequencing | Turnaround time (TAT) | Therapy/ clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|---|---|
| ARUP Laboratories Salt Lake City, Utah, USA | Solid Tumor Mutation Panel by Next Gneration Sequencing | DNA | 48 genes (mutation hotspots) | Amplicon-based NGS | No | 12–14 days | No | 81445 | http://ltd.aruplab.com/Tests/Pub/2007991 |
| Baylor Miraca Genetics Laboratories Houston, TX, USA | Solid Tumor Mutation Panel | DNA | 50 genes (targeted regions) | Amplicon-based, Ion AmpliSeq™ technology | No | 14 days | No | 81445 | https://www.bcm.edu/research/medical-genetics-labs/test_detail.cfm?testcode=9705 |
| Cancer Genetics Inc. Rutherford, NJ, USA | Focus::Oncomine™ | DNA | 35 genes (mutation hotspots) | Amplicon-based, Ion PGM platform | No | 7–10 days | No | 81445 | http://www.cancergenetics.com/wordpress/wp-content/uploads/2016/05/Focus-Oncomine-05.06.16.pdf |
| Caris Life Sciences Irving, TX, USA | Molecular Intelligence® Tumor Profiling | DNA and RNA | 46- and 592-gene panels (616 biomarkers) | NGS via Illumina (NextSeq™), RNA-seq, pyro sequencing, qPCR, MSI fragment analysis, IHC, CISH, FISH | No | 10–14 days | Yes | Not specified | https://www.carismolecularintelligence.com/tumor-profiling-menu/mi-profile-usa-excluding-new-york/ |
| Centogene AG Rostock, Germany | Cancer Hotspot Panel | DNA | 49 genes (mutation hotspots) | Amplicon-based, Ion PGM platform | No | 10 days | No | 81445 | https://www.centogene.com/centogene/centogene-test-catalogue-detail.php?test=NGS&ID=589b1c46&search=Panel&disease=Cancer_Hotspot_Panel |
| | Solid Tumor Panel | DNA | 63 genes (96% of coding regions) | Amplicon-based, Ion Proton platform | No | 10 days | No | 81455 | https://www.centogene.com/centogene/centogene-test-catalogue-detail.php?test=NGS&ID=d82e9f6d&search=Panel |
| Foundation Medicine, Inc. Cambridge, MA, USA | FoundationOne™ | DNA | 315 genes (coding sequence), introns of 28 genes for rearrangements | Hybrid-capture, Illumina (HiSeq™) | No | ≤14 days | Yes | Not specified | http://foundationone.com/learn.php?__hstc=231517312.33390d1810c250fde1b020446b793cc2.1477401945588.1483910263074.1484784265027.5&__hssc=231517312.1.1484784265027&__hsfp=3393377187#2 |
| | FoundationOne™ Heme (includes ematologic malignancies, sarcomas, and pediatric cancers) | DNA and RNA | 406 genes (coding sequence), rearrangements for 31 genes, 265 gene fusion partners | Hybrid-capture, Illumina (HiSeq™) | No | 12–14 days | Yes | Not specified | http://foundationone.com/learn.php?__hstc=231517312.33390d1810c250fde1b020446b793cc2.1477401945588.1483910263074.1484784265027.5&__hssc=231517312.1.1484784265027&__hsfp=3393377187#2 |
| Genoptix, Inc. | NexCourse® Complete | DNA | 173 genes | Hybrid-capture NGS | No | 12 days | No | 81455 (88381 if applicable) | |

**Table 3** (continued)

| Company/ laboratory | Test name | DNA/ RNA | Panel details | Methodology | Tumor/ normal sequencing | Turnaround time (TAT) | Therapy/ clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|---|---|
| Carlsbad, CA, USA | (includes solid tumors and hematologic malignancies) | | | | | | | | https://www.genoptix.com/sites/www.genoptix.com/files/uploads/files/Commercial%20Testing%20Directory%20(101016).pdf |
| | NexCourse® Solid | DNA | 110 genes (targeted regions) | Hybrid-capture NGS | No | 12 days | No | 81455 (88381 if applicable) | https://www.genoptix.com/sites/www.genoptix.com/files/uploads/files/Commercial%20Testing%20Directory%20%28101016%29.pdf |
| GenPath Diagnostics Elmwood, NJ, USA | OnkoSight™ for Solid Tumors | DNA | 31 genes (targeted regions) | Illumina | No | 5–10 days | Yes | 81210, 81235, 81403 (3), 81272, 81275, 81276, 81311, 81314, 81404 (2), 81405 | http://www.genpathdiagnostics.com/oncology/tests/?test_id=31fcc47b-88da-8df9-87aa-5637b3a1ad4a |
| Jackson Laboratory Farmington, CT, USA | ActionSeq™ | DNA | 212 genes | Hybrid-capture, Illumina | No | Not specified | Yes | Not specified | https://www.jax.org/clinical-genomics/clinical-offerings/actionseq |
| | JAX Cancer Treatment Profile™ | DNA and R-NA | 358 genes (coding sequence), 53 gene fusion partners | Hybrid-capture, Illumina; Archer™ fusion detection | No | Not specified | Yes | Not specified | https://www.jax.org/clinical-genomics/clinical-offerings/jax-cancer-treatment-profile |
| Kew, Inc. Cambridge, MA, USA | CANCERPLEX® TX (FDA-approved targeted therapies only) | DNA | 67 genes (coding sequence) | Hybrid-capture, Illumina | No | 7–10 days after receiving specimen | Yes | Not specified | http://kewinc.com/cancerplex/cancerplex-tx/ |
| | CANCERPLEX® FP + IO (Full Panel with detection of hypermutated phenotype) | DNA | 413 genes (coding sequence), translocation detection for 19 genes (partial introns), detection of EBV and HPV16/18 | Hybrid-capture, Illumina | No | 7–10 days after receiving specimen | Yes | Not specified | http://kewinc.com/cancerplex/cancerplex-fp/ |
| LabCorp Burlington, NC, USA | IntelliGen® | DNA | 50 genes (mutation hotspots) | Amplicon-based | No | Not specified | Yes | 81445, 88381 | https://www.labcorp.com/test-menu/32266/oncology-therapeutic-panel-intelligen%C2%AE |
| Mayo Medical Laboratories Rochester, MN, USA | CAPN/Solid Tumor Targeted Cancer Gene Panel by NGS | DNA | 50 genes (targeted regions) | PCR-based NGS | No | ≤20 days | Yes | 81445, 88381 | http://www.mayomedicallaboratories.com/test-catalog/Overview/35594 |
| NeoGenomics Laboratories, Inc. Fort Myers, FL, USA | NeoType™ Precision Profile for Solid Tumors | DNA | 48 genes, PD-L1 IHC | NGS, IHC | No | 14 days | No | 81210, 81245, 81275, 81276 × 2, 81310, 81311, 81321, 81403 × 4, 81445, 88360, G0452 | http://neogenomics.com/test-menu/neotype-precision-profile-for-solid-tumors |
| | | DNA | | NGS, FISH, IHC | No | 22 days | No | | |

**Table 3** (continued)

| Company/ laboratory | Test name | DNA/ RNA | Panel details | Methodology | Tumor/ normal sequencing | Turnaround time (TAT) | Therapy/ clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|---|---|
| | NeoTYPE™ Discovery Profile for Solid Tumors | | 315 genes (coding sequence), PD-L1 IHC, 9 FISH | | | | | 81455, 88342, 88374, 88377 × 8, G0452 (88374 × 1 automated HER2 FISH or 88377 × 1 for manual, if applicable) | http://neogenomics.com/Test-Menu/neotype-discovery-profile-for-solid-tumors |
| OncoDNA SA Gosselies, Belgium | OncoDEEP™ | DNA and R-NA | 65 genes (mutation hotspots) | NGS, IHC, other (translocation analysis, methylation) | No | 7 days | Yes | Not specified | http://www.oncodna.com/solutions/oncodeep |
| | OncoSTRAT&GO™ | DNA and R-NA | >150 genes (mutations hotspots), >350 gene fusions, selection of up to 15 variants for further monitoring with OncoTRACE | NGS, IHC, other (translocation analysis, unusual splicing, methylation) | No | 10 days | Yes | Not specified | http://www.oncodna.com/solutions/oncodeep |
| Paradigm Phoenix, AZ, USA | Paradigm Cancer Diagnostic (PCDx) Panel | DNA and R-NA | 131 genes, 23 protein targets | Amplicon-based, Ion AmpliSeq™ technology, mRNA and protein expression by IHC | No | 4–5 days | Yes | Not specified | http://www.paradigmdx.com/wp-content/uploads/2016/08/PD10196PCDxTechnicalDoc2016-8-11.pdf |
| PathGroup Brentwood, TN, USA | SmartGenomics™ | DNA | 62 genes (mutation hotspots) | NGS, cytogenomic array | No | 7–10 days | Yes | Not specified | http://www.pathgroup.com/oncology/smartgenomics/ |
| Personal Genome Diagnostic-s, Inc. Baltimore, MD, USA | CancerSELECT™ R125 | DNA | 117 genes (coding sequence), 41 genes (selected regions) | Hybrid-capture, Illumina | Yes | 3 weeks | Yes | Not specified | http://www.personalgenome.com/research-services/tissue/ |
| Personalis, Inc. Menlo Park, CA, USA | ACE CancerPlus™ Test | DNA and R-NA | 181 genes (including fusions) | NGS | No | 3 weeks | Yes | Not specified | http://www.personalis.com/ace-cancerplus-test/ |
| Quest Diagnostic-s™ Madison, NJ, USA | OncoVantage® Solid Tumor Mutation Analysis | DNA | 34 genes (targeted regions) | Amplicon-based, Ion AmpliSeq™ technology | No | Not specified | Yes | 81445 | http://www.questdiagnostics.com/testcenter/testguide.action?dc=TS_OncoVantage |
| Rosetta Genomics Philadelphia, PA, USA | OncoGxOne™ | DNA | 64 cancer genes | NGS | No | 7–10 days | No | Not specified | http://rosettagx.com/testing-services/next-gen |
| | StrandAdvantage | DNA | | NGS | No | 3–4 weeks | Yes | Not specified | http://strandls.com/somatic-tests/ |

**Table 3** (continued)

| Company/ laboratory | Test name | DNA/ RNA | Panel details | Methodology | Tumor/ normal sequencing | Turnaround time (TAT) | Therapy/ clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|---|---|
| Strand Genomics, Inc. Hebbal, Bangalore, India | StrandAdvantage | DNA | 48 genes (hotspot regions)<br>152 genes (including rearrangements and fusions) | NGS | No | 6–8 weeks | Yes | Not specified | http://strandls.com/somatic-tests/ |

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sequencing (WGS), which could theoretically allow better detection of non-coding and structural changes, is even further away from clinical application. WES/WGS also generates an excess of information of questionable clinical utility, highlighting our inability to interpret the functional and clinical impact of any variant that has not been well studied in the literature. Demonstrating the significance of each and every variant, however, may not be practical or even feasible. Many variants are exceedingly rare, so to find enough patients to run a clinical trial to determine whether they are significant is next to impossible given the inconsistent application of tumor profiling by clinicians, variability of assays performed, and barriers to consenting patients for public data sharing.

Although the clinical utility of large scale sequencing in patients may be low today, such information could be extremely important in the future for researchers who want to interrogate these real-world data repositories to get prognostic information. Real-world data can not only provide information on how a drug compares to the standard of care actually used in clinical practice versus the comparator testing in a clinical trial, but provide a clearer picture of realistic endpoints that clinicians can readily understand. For example, time to next treatment or change in therapy and the reason for that change, such as progression or drug toxicity, is likely to be noted in medical records and a more relevant endpoint than progression-free survival outcomes. Standardizing existing clinical datasets and integrating them with real-world data streams can speed the development of new drugs or expand labeled indications while also reducing costs of development. If leveraged correctly, information gathered from electronic health records and patient registries can allow for data to be collected on more patients and in unselected patient populations. Although randomized controlled trials are still necessary to discern small treatment effects from other factors, studies using real-world evidence can be used for therapies that demonstrate large treatment effects in relatively heterogeneous patient populations.

Efforts to standardize variant interpretation and unify independently curated (and sometimes conflicting) variant databases with health and disease-related outcomes data is ongoing [6, 14, 16, 22, 23, 29, 33]. Databases of genetic variants have the potential to speed evidence development for multiplex testing, since they are typically generated by multiple sources. Additionally, aggregated data can also provide a stronger evidence base for multiplex testing in the real-world than any single clinical trial can produce. As understanding of cancer pathogenesis progresses, the classification of each variant relative to the reference genome and population-based databases will hopefully become more refined.

Cancer, however, is multifactorial and multigenic, involving not only random genomic alterations and environmental modifiers, but epigenetic and pleiotropic phenomena as well. Intra- and intertumoral genetic heterogeneity complicates the

**Table 4** Select laboratories offering comprehensive cancer testing with >1000 genes (compiled as of March 6, 2017)

| Company | Test name | Panel details | Tumor/normal sequencing | Turnaround time (TAT) | Therapy/clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|
| Columbia University New York, NY | Cancer Whole Exome with Transcriptome (cWES) | Whole exome sequencing<br>Whole transcriptome sequencing | Yes | 60 days | Not specified | 81201, 81216, 81292, 81295, 81298, 81321, 81275, 81235, 81210, 81245, 81310, 81403, 81404, 81405, 81406, 81407, 81408 | http://pathology.columbia.edu/diagnostic/PGM/oncologytests.html#CWES |
| Jefferson Cancer Genomics Laboratory Philadelphia, PA | Whole Exome Sequencing (WES) | Whole exome sequencing | Not specified | 6 weeks | Not specified | Not specified | http://www.jefferson.edu/university/jmc/departments/cancer-biology/research/cancer-genomics/services.html |
| NeoGenomics Laboratories, Inc. Fort Myers, FL | NeoTYPE™ Cancer Exome Profile | 4813 genes (coding regions) | Not specified | 21 days | Not specified | 81455, G0452 | http://neogenomics.com/test-menu/neotype-cancer-exome-profile |
| | Pan-Cancer NGS Expression/Fusion Panel | Targeted NGS RNA sequencing to detect all fusion transcripts in 1385 genes | Not specified | 21 days | Not specified | 81455 | http://neogenomics.com/test-menu/pan-cancer-ngs-expression-fusion-panel |
| NantHealth, Inc. Culver City, CA | GPS Cancer™ | Whole genome sequencing<br>Whole transcriptome sequencing<br>Protein expression analysis by targeted mass spectrometry | Yes | <21 days | Yes | Not specified | http://www.gpscancer.com/overview/ |
| Personal Genome Diagnostics, Inc. Baltimore, MD | CancerComplete® | Whole exome sequencing | Yes | 6 weeks | Yes | Not specified | http://www.personalgenome.com/research-services/tissue/ |

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**Table 5** Select laboratories offering liquid biopsy tests (compiled as of March 6, 2017)

| Company | Test name | cfDNA or RNA | Panel details | Methodology | Turnaround time (TAT) | Therapy/clinical trial matching | CPT® code(s) | URL source |
|---|---|---|---|---|---|---|---|---|
| Foundation Medicine, Inc. Cambridge, MA, USA | FoundationACT™ | cfDNA | 62 genes (all clinically relevant genomic alterations), fusions for 6 genes | Hybrid-capture | ≤14 days | Yes | Not specified | http://foundationone.com/docs/FoundationACT/FM-ACT_TechnicalSpecsOverview_FINAL.pdf?__hstc=197910000.f4823a40614c9b44882d24964bb139bf.1475497940453.1478795333311.1481140065350.20&__hssc=197910000.2.1481140065350&__hsfp=3109191104 |
| Genomic Health, Inc. Redwood City, CA, USA | Oncotype SEQ® Liquid Select | cfDNA | 17 genes | Not specified | ≤14 days | Yes | Not specified | http://www.oncotypeseq.com/liquid-select |
| | Guardant360 | cfDNA | 73 genes (partial or full coding regions, 1 select promoter region), fusions for 6 genes | Hybrid-capture, Illumina (HiSeq™) | 2 weeks | Yes | Not specified | http://www.guardanthealth.com/ |
| NeoGenomics Laboratories, Inc. Fort Myers, FL, USA | NeoLAB™ Solid Tumor Monitor Liquid Biopsy | cfDNA or RNA | 48 genes | NGS | 14 days | Not specified | 81445, G04-52 | http://neogenomics.com/test-menu/neolab-solid-tumor-monitor |
| OncoDNA SA Gosselies, Belgium | OncoTrace™ | cfDNA | 28 genes (hotspot mutations) and monitoring of up to 15 previously identified variants | NGS | 7 days | Yes | Not specified | http://www.oncodna.com/solutions/oncotrace |
| Pathway Genomics San Diego, CA, USA | CancerIntercept™ Monitor | cfDNA | 9 genes (mutation hotspots) | Amplicon-based, Illumina | 2–3 weeks | Optional | Not specified | https://www.pathway.com/cancer-intercept-monitor/ |
| Personal Genome Diagnostics, Inc. Baltimore, MD | PlasmaSELECT™ | cfDNA | 58 genes (full coding and specific exon analysis), rearrangements for 17 genes | Hybrid-capture, Illumina | 2–3 weeks | Not specified | Not specified | http://www.personalgenome.com/wp-content/uploads/2016/10/PGD_PlasmaSELECT_Bulletin.pdf |

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task of identifying alterations that drive tumor progression from those that do not, and regional differences within a tumor that are further exaggerated by different selective pressures (e.g., variable access to oxygen, differences in tumor microenvironment, inconsistent exposure to targeted and non-targeted cancer therapies). These factors also need to be interpreted within the context of normal human genomic variation [13, 19, 20, 24, 30], which is an inherent part of somatic interpretation.

Since there are still many "dark" regions of the genome yet to be characterized or understood [2, 25], a reasonable approach for clinical laboratories looking to improve precision medicine efforts may be the adoption of larger pan-cancer tests with whole-gene sequencing. Memorial Sloan Kettering Cancer Center (MSKCC) and the Dana-Farber Institute/Brigham and Women's Hospital (DFI/BWH) have created robust research infrastructures around initiatives that prioritize sequencing of all cancer patients with large custom-designed panels (approximately 300 to 500 genes) at their respective institutions [18, 34]. These initiatives have allowed every clinical patient, regardless of age, stage, or tumor type, to contribute to our understanding of cancer, particularly in genes not commonly sequenced as part of current standard of care guidelines. The size of these panels has not only facilitated novel discovery, but more importantly, provided a broad enough landscape to simultaneously assess tumor mutational burden, copy number variation, and tumor subclones. Along with public data sharing of outcomes data, more efforts like these would greatly accelerate cancer research and therapeutic development. Independence Blue Cross of Philadelphia, a private insurance provider, has also embraced the unique paradigm of comprehensive tumor profiling in the clinical setting for the purposes of discovery by offering coverage of NantHealth's GPC Cancer™ test (Table 4) [3]. This test includes not only whole exome and transcriptome sequencing, but also comparative germline testing and quantitative proteomics by mass spectrometry. Complex multiomic data generation not only contributes to our collective understanding of tumor biology, but also provides rationale for combination therapies that target complex biologic processes.

Recent clinical sequencing reports from various groups [1, 26] point to the value of incorporating RNA sequencing (RNA-seq) with DNA sequencing to evaluate the expression of mutant alleles, to detect both known and novel gene fusions, and to confirm splice variants. RNA-seq may be especially valuable in pediatric tumors, which tend to have fewer recurrent point mutations compared with adult tumors. One case series of young patients showed that RNA-seq alone accounted for approximately 20% of actionable findings which would have been missed with WES [26]. Integrated RNA and DNA sequencing strategies may also aid in the identification of patient-specific immunogenic neoantigens expressed in the tumors which are becoming increasingly relevant for personalized cancer vaccine development [15].

Newer testing methods, such as liquid biopsies for solid tumors, have the advantage of being non-invasive and do not suffer from the same type of sample bias associated with tissue biopsies. This technology is still in its infancy and is limited to the detection of metastatic disease burden based on known molecular status of the primary tumor [17]. Nevertheless, liquid biopsy testing is slowly becoming available for patients who are not candidates for tissue biopsy and are at a high risk for resistance mutations to targeted therapy (Table 5). It is likely that this technology will not replace tissue-based testing, but 1 day may be used for minimal residual disease monitoring in a subset of patients with canonical tumor alterations.

The future of precision oncology lies in the detailed molecular characterization of a large number of patients, across ethnic, socioeconomic, and geographic subgroups, and prospective linking of multiplex testing results with individual clinical data [31]. Such concerted efforts, supported by government and private payers, will revolutionize genetic discovery, furthering our knowledge for all cancers and improving the care of patients with molecularly defined cancer in the generations to come.

Although it remains to be seen how clinical interpretation, annotation, and integration of such complex molecular data will evolve [28], broad application of standardized tumor profiling, irrespective of whether molecular testing is required to guide standard-of-care therapy, is needed to lay the groundwork for more systematic real-world studies.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW (2016) Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nat Rev Genet 17:257–271. doi:10.1038/nrg.2016.10
2. Chi KR (2016) The dark side of the human genome. Nature 538: 275–277. doi:10.1038/538275a
3. CMTP (2017) New genomic technologies in clinical medicine: current evidence and decision-making http://www.cmtpnet.org/docs/resources/Genomic_Forum_Meeting_Briefing.pdf. Accessed May 9, 2017
4. Collins FS, Green ED, Guttmacher AE, Guyer MS, Institute UNHGR (2003) A vision for the future of genomics research. Nature 422:835–847. doi:10.1038/nature01626

5. Consortium IHGS (2004) Finishing the euchromatic sequence of the human genome. Nature 431:931–945. doi:10.1038/nature03001
6. Doig KD, Fellowes A, Bell AH, Seleznev A, Ma D, Ellul J, Li J, Doyle MA, Thompson ER, Kumar A, Lara L, Vedururu R, Reid G, Conway T, Papenfuss AT, Fox SB (2017) PathOS: a decision support system for reporting high throughput sequencing of cancers in clinical diagnostic laboratories. Genome Med 9:38. doi:10.1186/s13073-017-0427-z
7. Engstrom PF, Bloom MG, Demetri GD, Febbo PG, Goeckeler W, Ladanyi M, Loy B, Murphy K, Nerenberg M, Papagni P, Robson M, Sweetman RW, Tunis S, Demartino J, Larsen JK, Network NCC (2011) NCCN molecular testing white paper: effectiveness, efficiency, and reimbursement. J Natl Compr Cancer Netw 9(Suppl 6):S1–16
8. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L, Komaki R, Lackner RP, Lanuti M, Leal TA, Leisch LJ, Lilenbaum R, Lin J, Loo BW, Martins R, Otterson GA, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Stevenson J, Swanson SJ, Tauer K, Yang SC, Gregory K, Hughes M (2017) Non-small cell lung cancer, version 5.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 15:504–535
9. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L, Komaki R, Lackner RP, Lanuti M, Leal TA, Leisch LJ, Lilenbaum R, Lin J, Loo BW, Martins R, Otterson GA, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Stevenson J, Swanson SJ, Tauer K, Yang SC, Gregory K, Hughes M (2017) Non-small cell lung cancer, version 6.2017, NCCN Clinical Practice Guidelines in Oncology. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed May 21, 2017
10. FDA Hematology/Oncology (Cancer) Approvals & Safety Notifications. https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm279174.htm. Accessed May 25, 2017
11. Febbo PG, Ladanyi M, Aldape KD, De Marzo AM, Hammond ME, Hayes DF, Iafrate AJ, Kelley RK, Marcucci G, Ogino S, Pao W, Sgroi DC, Birkeland ML (2011) NCCN Task Force report: evaluating the clinical utility of tumor markers in oncology. J Natl Compr Canc Netw 9 Suppl 5:S1–32 quiz S33
12. Garcia EP, Minkovsky A, Jia Y, Ducar MD, Shivdasani P, Gong X, Ligon AH, Sholl LM, Kuo FC, MacConaill LE, Lindeman NI, Dong F (2017) Validation of OncoPanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer doi:10.5858/arpa.2016-0527-OA.
13. Garofalo A, Sholl L, Reardon B, Taylor-Weiner A, Amin-Mansour A, Miao D, Liu D, Oliver N, MacConaill L, Ducar M, Rojas-Rudilla V, Giannakis M, Ghazani A, Gray S, Janne P, Garber J, Joffe S, Lindeman N, Wagle N, Garraway LA, Van Allen EM (2016) The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. Genome Med 8:79. doi:10.1186/s13073-016-0333-9
14. Griffith M, Spies NC, Krysiak K, McMichael JF, Coffman AC, Danos AM, Ainscough BJ, Ramirez CA, Rieke DT, Kujan L, Barnell EK, Wagner AH, Skidmore ZL, Wollam A, Liu CJ, Jones MR, Bilski RL, Lesurf R, Feng YY, Shah NM, Bonakdar M, Trani L, Matlock M, Ramu A, Campbell KM, Spies GC, Graubert AP, Gangavarapu K, Eldred JM, Larson DE, Walker JR, Good BM, Wu C, Su AI, Dienstmann R, Margolin AA, Tamborero D, Lopez-Bigas N, Jones SJ, Bose R, Spencer DH, Wartman LD, Wilson RK, Mardis ER, Griffith OL (2017) CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. Nat Genet 49:170–174. doi:10.1038/ng.3774
15. Gupta S, Chaudhary K, Dhanda SK, Kumar R, Kumar S, Sehgal M, Nagpal G, Raghava GP (2016) A platform for designing genome-based personalized immunotherapy or vaccine against cancer. PLoS One 11:e0166372. doi:10.1371/journal.pone.0166372
16. Harrison SM, Dolinsky JS, Knight Johnson AE, Pesaran T, Azzariti DR, Bale S, Chao EC, Das S, Vincent L, Rehm HL (2017) Clinical laboratories collaborate to resolve differences in variant interpretations submitted to ClinVar. Genet Med. doi:10.1038/gim.2017.14
17. Hofman P, Popper HH (2016) Pathologists and liquid biopsies: to be or not to be? Virchows Arch 469:601–609. doi:10.1007/s00428-016-2004-z
18. Hyman DM, Solit DB, Arcila ME, Cheng DT, Sabbatini P, Baselga J, Berger MF, Ladanyi M (2015) Precision medicine at Memorial Sloan Kettering Cancer Center: clinical next-generation sequencing enabling next-generation targeted therapy trials. Drug Discov Today 20:1422–1428. doi:10.1016/j.drudis.2015.08.005
19. Jones S, Anagnostou V, Lytle K, Parpart-Li S, Nesselbush M, Riley DR, Shukla M, Chesnick B, Kadan M, Papp E, Galens KG, Murphy D, Zhang T, Kann L, Sausen M, Angiuoli SV, Diaz LA, Velculescu VE (2015) Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med 7:283ra253. doi:10.1126/scitranslmed.aaa7161
20. Kline CN, Joseph NM, Grenert JP, van Ziffle J, Talevich E, Onodera C, Aboian M, Cha S, Raleigh DR, Braunstein S, Torkildson J, Samuel D, Bloomer M, Campomanes AG, Banerjee A, Butowski N, Raffel C, Tihan T, Bollen AW, Phillips JJ, Korn WM, Yeh I, Bastian BC, Gupta N, Mueller S, Perry A, Nicolaides T, Solomon DA (2016) Targeted next-generation sequencing of pediatric neuro-oncology patients improves diagnosis, identifies pathogenic germline mutations, and directs targeted therapy. Neuro-Oncology. doi:10.1093/neuonc/now254
21. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann Y, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blöcker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M,

Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowki J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ, Consortium IHGS (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921. doi:10.1038/35057062

22. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, Jang W, Katz K, Ovetsky M, Riley G, Sethi A, Tully R, Villamarin-Salomon R, Rubinstein W, Maglott DR (2017) ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res 44:D862–D868. doi:10.1093/nar/gkv1222
23. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimberidou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN (2017) Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 19:4–23. doi:10.1016/j.jmoldx.2016.10.002
24. Luthra R, Patel KP, Routbort MJ, Broaddus RR, Yau J, Simien C, Chen W, Hatfield DZ, Medeiros LJ, Singh RR (2016) A targeted high-throughput next-generation sequencing panel for clinical screening of mutations, gene amplifications, and fusions in solid tumors. J Mol Diagn. doi:10.1016/j.jmoldx.2016.09.011
25. Martin L, Chang HY (2012) Uncovering the role of genomic "dark matter" in human disease. J Clin Invest 122:1589–1595. doi:10.1172/JCI60020
26. Mody RJ, Wu YM, Lonigro RJ, Cao X, Roychowdhury S, Vats P, Frank KM, Prensner JR, Asangani I, Palanisamy N, Dillman JR, Rabah RM, Kunju LP, Everett J, Raymond VM, Ning Y, Su F, Wang R, Stoffel EM, Innis JW, Roberts JS, Robertson PL, Yanik G, Chamdin A, Connelly JA, Choi S, Harris AC, Kitko C, Rao RJ, Levine JE, Castle VP, Hutchinson RJ, Talpaz M, Robinson DR, Chinnaiyan AM (2015) Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. JAMA 314:913–925. doi:10.1001/jama.2015.10080
27. Myers RE, Wolf T, Shwae P, Hegarty S, Peiper SC, Waldman SA (2016) A survey of physician receptivity to molecular diagnostic testing and readiness to act on results for early-stage colon cancer patients. BMC Cancer 16:766. doi:10.1186/s12885-016-2812-1
28. Nakagawa H, Wardell CP, Furuta M, Taniguchi H, Fujimoto A (2015) Cancer whole-genome sequencing: present and future. Oncogene 34:5943–5950. doi:10.1038/onc.2015.90
29. Ritter DI, Roychowdhury S, Roy A, Rao S, Landrum MJ, Sonkin D, Shekar M, Davis CF, Hart RK, Micheel C, Weaver M, Van Allen EM, Parsons DW, McLeod HL, Watson MS, Plon SE, Kulkarni S, Madhavan S, Group CSCW (2016) Somatic cancer variant curation and harmonization through consensus minimum variant level data. Genome Med 8:117. doi:10.1186/s13073-016-0367-z
30. Schrader KA, Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, Ni A, Thomas T, Benayed R, Ashraf A, Lincoln A, Arcila M, Stadler Z, Solit D, Hyman DM, Hyman D, Zhang L, Klimstra D, Ladanyi M, Offit K, Berger M, Robson M (2016) Germline Variants in Targeted Tumor Sequencing Using Matched Normal DNA. JAMA Oncol 2:104–111. doi:10.1001/jamaoncol.2015.5208
31. Sholl LM, Do K, Shivdasani P, Cerami E, Dubuc AM, Kuo FC, Garcia EP, Jia Y, Davineni P, Abo RP, Pugh TJ, van Hummelen P, Thorner AR, Ducar M, Berger AH, Nishino M, Janeway KA, Church A, Harris M, Ritterhouse LL, Campbell JD, Rojas-Rudilla V, Ligon AH, Ramkissoon S, Cleary JM, Matulonis U, Oxnard GR, Chao R, Tassell V, Christensen J, Hahn WC, Kantoff PW, Kwiatkowski DJ, Johnson BE, Meyerson M, Garraway LA, Shapiro GI, Rollins BJ, Lindeman NI, MacConaill LE (2016) Institutional implementation of clinical tumor profiling on an unselected cancer population. JCI Insight 1:e87062. doi:10.1172/jci.insight.87062
32. Singh RR, Patel KP, Routbort MJ, Reddy NG, Barkoh BA, Handal B, Kanagal-Shamanna R, Greaves WO, Medeiros LJ, Aldape KD, Luthra R (2013) Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. J Mol Diagn 15:607–622. doi:10.1016/j.jmoldx.2013.05.003
33. Yen JL, Garcia S, Montana A, Harris J, Chervitz S, Morra M, West J, Chen R, Church DM (2017) A variant by any name: quantifying annotation discordance across tools and clinical databases. Genome Med 9:7. doi:10.1186/s13073-016-0396-7
34. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen H-W, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M, Berger MF (2017) Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. doi:10.1038/nm.4333 http://www.nature.com/nm/journal/vaop/ncurrent/abs/nm.4333.html#supplementary-information