

# Cancer Gene Panels

A 3D digital illustration of cancer cells. The central focus is a large, spherical cell with a highly textured, bumpy surface and several long, thin, hair-like projections extending from it. To its left is a smaller, more rounded cell with a smoother surface and a prominent, bright pink nucleus. The background is a dark red, textured surface that resembles a microscopic view of tissue or a network of fibers.

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- Founded in 1998
- Main outside investor: GSK
- Referenced by Gartner in the 2014 Hype Cycle for Life Sciences
- Providing analytics software for research and clinicians
- Thousands of users in hundreds of organizations world wide

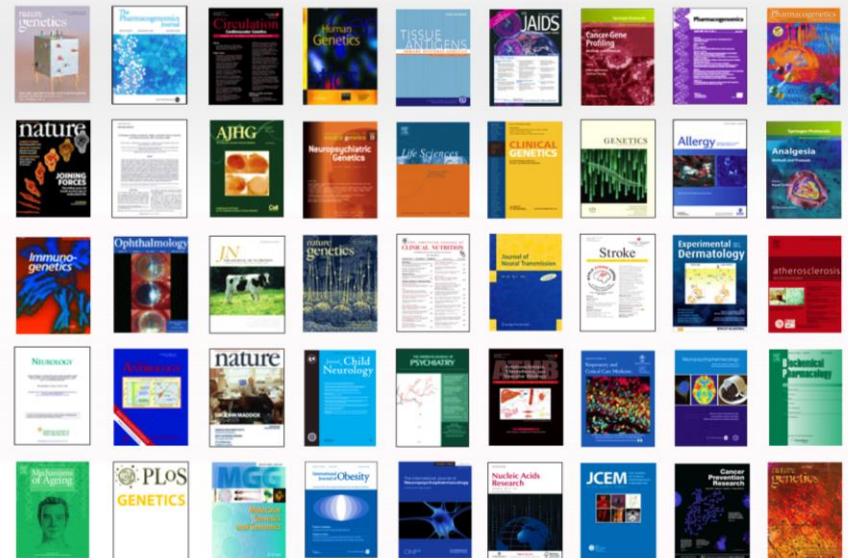
# About Golden Helix



## Hundreds of Customers Across the Globe



## Over 850 Citations



# Cancer is a disease of the genome!



Theodore Boveri (1862 – 1915)

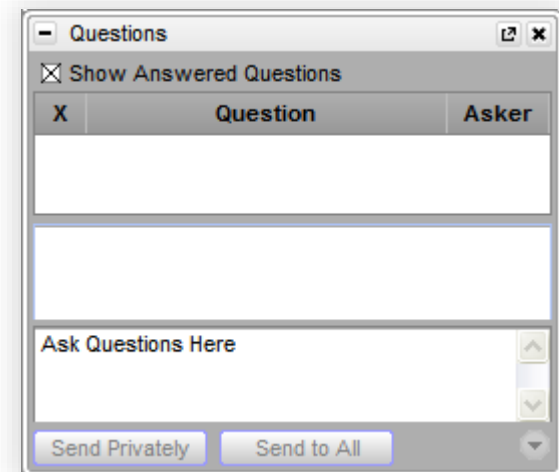


Sea Urchins



# Questions during the presentation

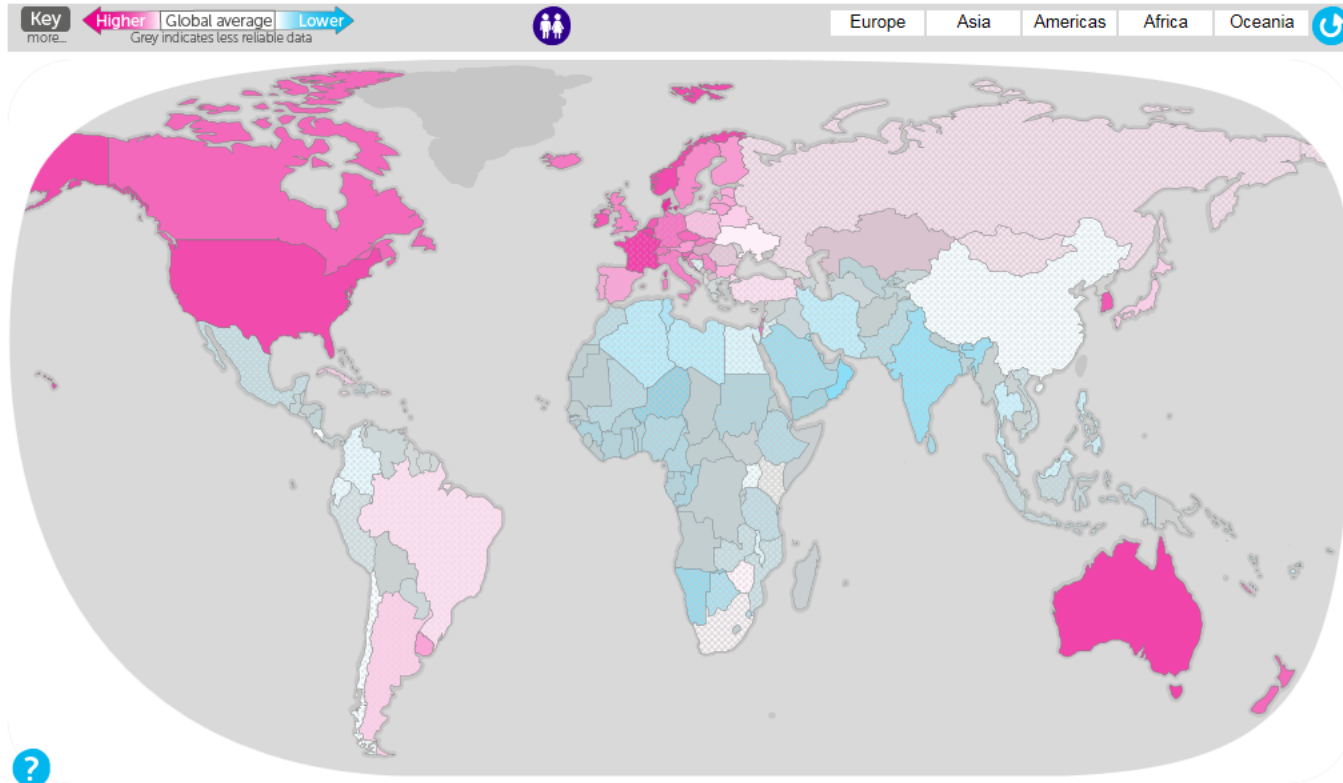
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# Global cancer incidence



## Cancer incidence — Worldwide

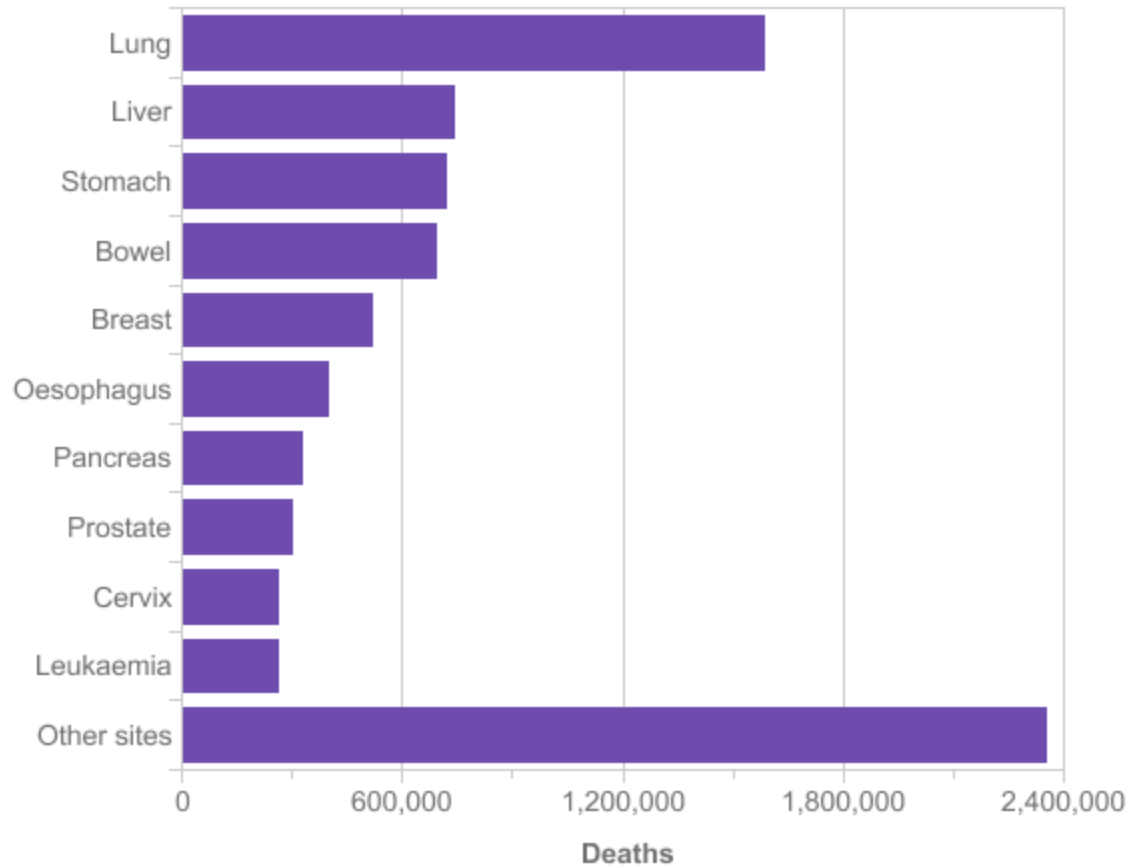


Worldwide cancer incidence — 14,090,149 cases per year:

Lung	Bowel	Liver	NHL	Thyroid	Myeloma	HL
			Leukaemia	Brain	Nasopharynx	Testis
	Prostate	Cervix	Pancreas	Ovary	Others	
Breast	Stomach	Oesophagus	Kidney	Melanoma		
		Bladder	Uterus	Gallbladder		
			Lip	Larynx		
				Pharynx		

<http://info.cancerresearchuk.org/cancerstats/faqs>

# Global mortality figures



<http://info.cancerresearchuk.org/cancerstats/faqs>

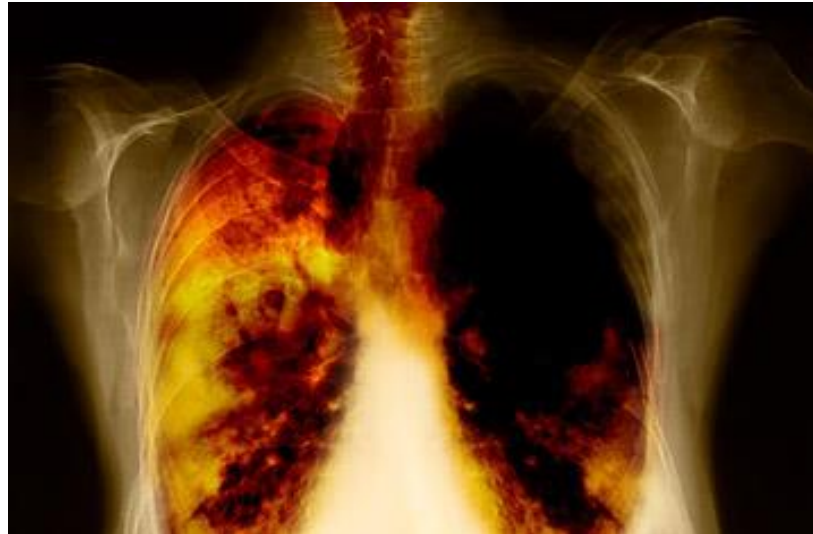


- In 2012 about 14.1 million cases in cancer occurred globally (excluding skin cancer). Common types are

Males	Females
Lung cancer	Breast cancer
Prostate cancer	Lung cancer
Colorectal cancer	Colorectal cancer
Stomach cancer	Cervical cancer

- Cancer risk increases with age. It occurs more commonly in the developed world due to increased life expectancy and lifestyle choices.
- The financial costs of cancer is estimated to be \$1.16 trillion in 2010 according to the World Cancer Report.





- Small cell lung cancer (SCLC): Highly aggressive with a high likelihood of metastases at diagnosis. Mostly, patients are treated with chemotherapy.
- Non-small cell lung cancer (NSCLC): About one third of the patients are diagnosed with this subtype. If caught early enough, then the likelihood of the cancer being local to the lungs is high. Therefore surgery is a valid treatment option, although the chances for NSCLS patients to develop recurrences after surgery is still to be quantified at 30%-60%.



**Crizotinib**



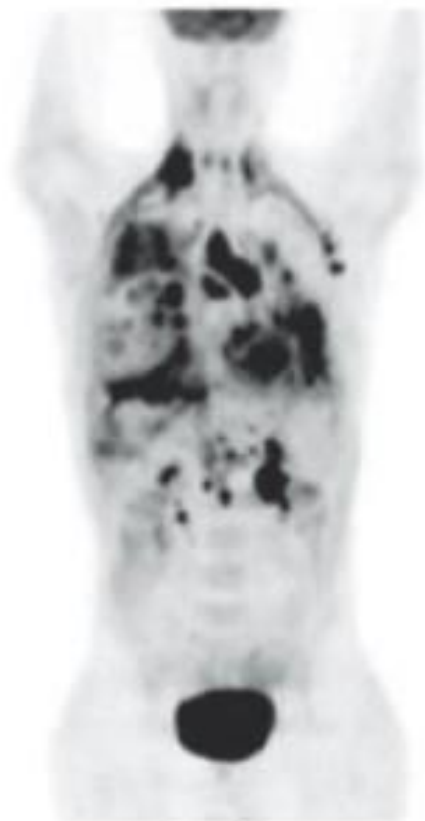
**Ceritinib**

- Now, in recent years more effective therapies have been developed to target very specific molecules or pathways that influence the cancer tumor. One example is the anaplastic lymphoma kinase (ALK). Clinical trials have shown that patients with tumors driven by these aberrant genes can be treated with very specific drugs resulting in response rates of over 60%.
- Craddock et. al. (2013) provides an extensive list of genes that have mutated forms linked to lung cancers. The variations are typically simple mutations that can be tested effectively via a gene panels

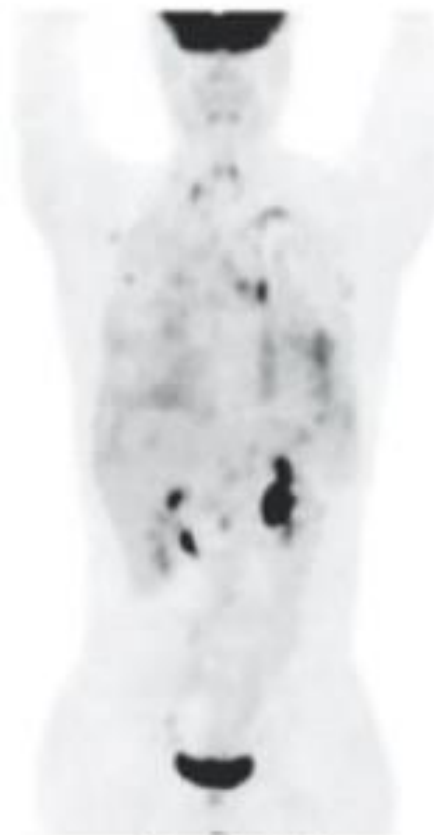
# Impact of Ceritinib



## Positron-Emission Tomographic Scans



Baseline



After 3.5 Wk

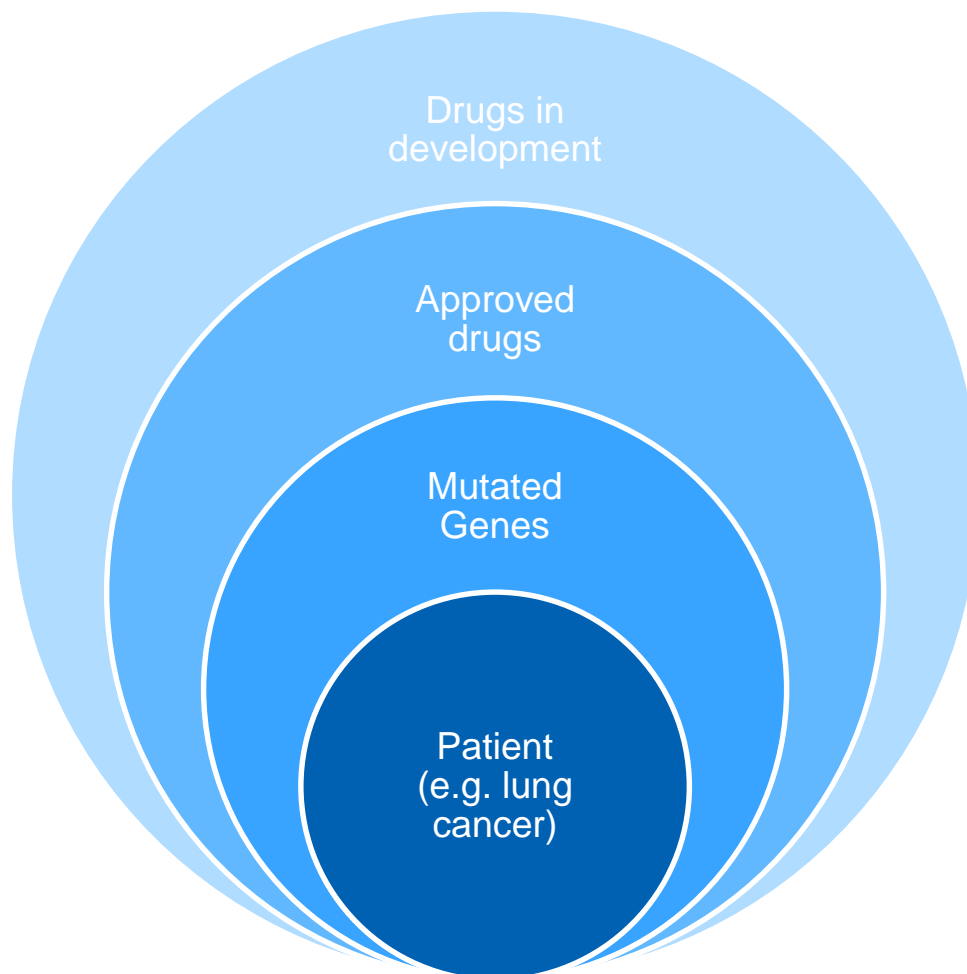
# Cost of testing in personalized medicine



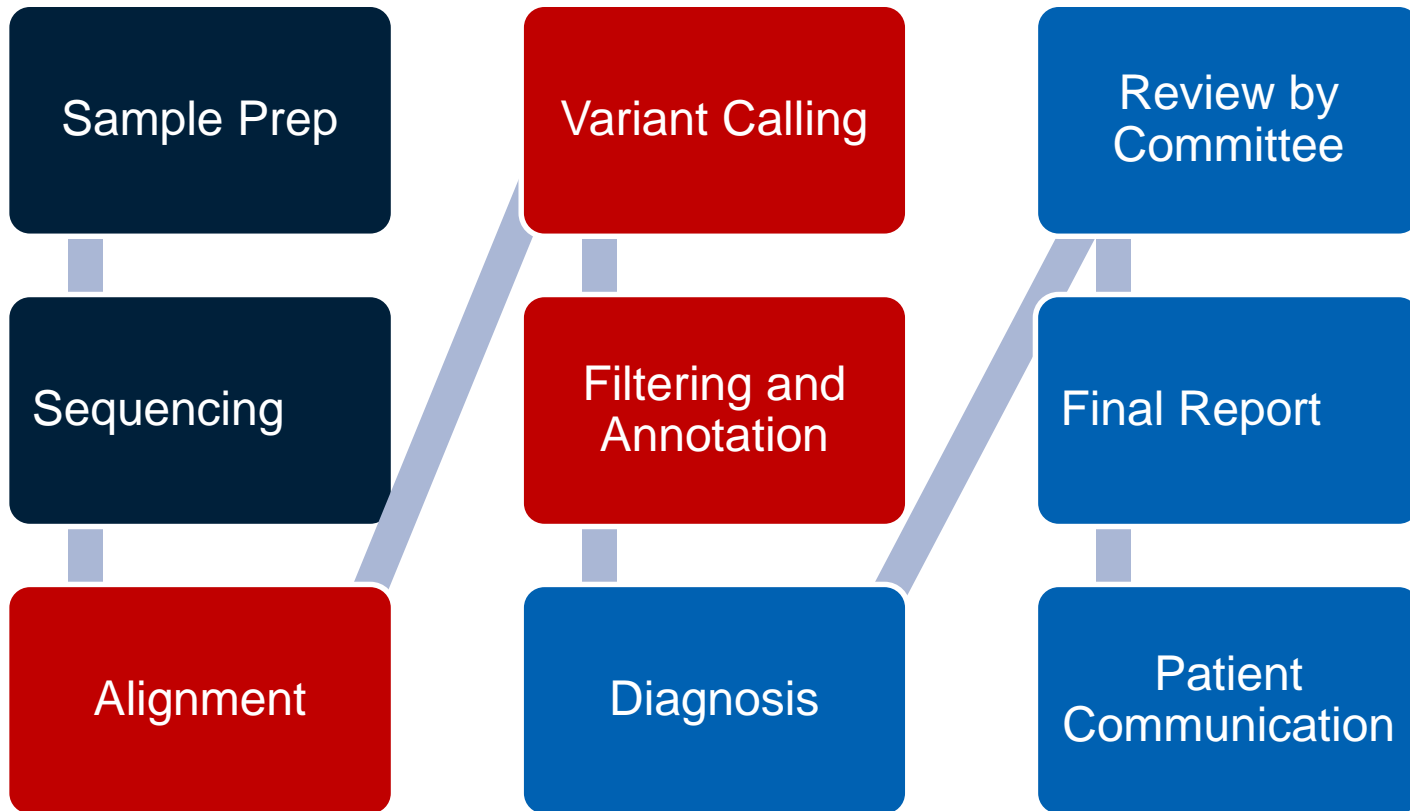
- Result of recent phase III study compared crizotinib with standard chemotherapy in patients with locally advanced or metastatic *ALK*-positive lung cancer:
  - median progression-free survival (PFS), 7.7 versus 3.0 months;
  - response rate: 65% versus 20%; and
  - symptoms and quality of life were also substantially better.
  - 64% of the group receiving chemotherapy crossed over to crizotinib.
- Besides the cost of the drug itself, one main question remains unanswered:
  - Can we afford to screen everyone with lung cancer, given that only 3% to 5% of the population will be *ALK* positive
  - Need to screen 100 patients to find approximately three who test positive. The only US Food and Drug Administration–approved test is the Vysis LSI *ALK* Break Apart FISH Probe Kit (Abbott Molecular), with near 100% accuracy but at a cost of more than \$250/test.

Kelly, R; Hillner, B; Smith, T. “Cost Effectiveness of Crizotinib of Anaplastic Lymphoma Kinase-Positive, Non-Small Cell Lung Cancer: Who is going to Blink at the costs?” , American Society of Clinical Oncology 2014

# Limitations of Companion Dx



# Cancer Gene Panel Workflow – An Overview



# Ion AmpliSeq Cancer Hotspot Panel

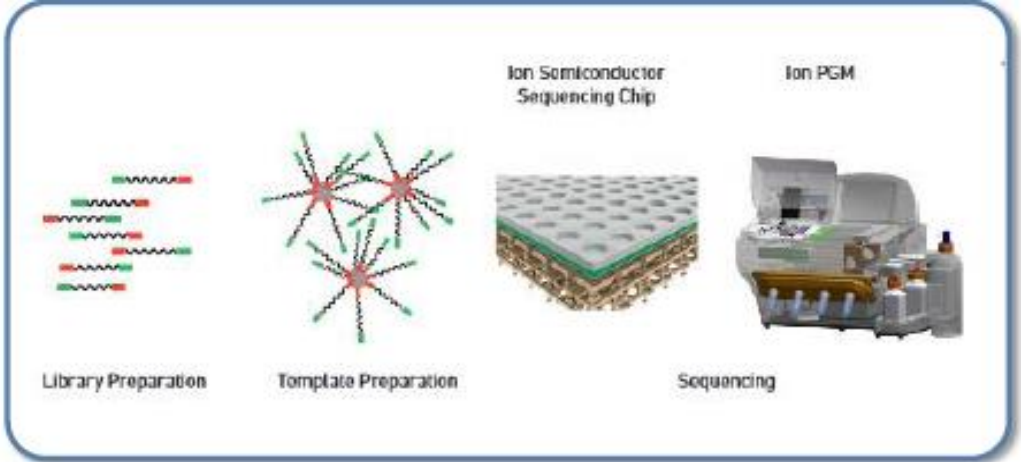
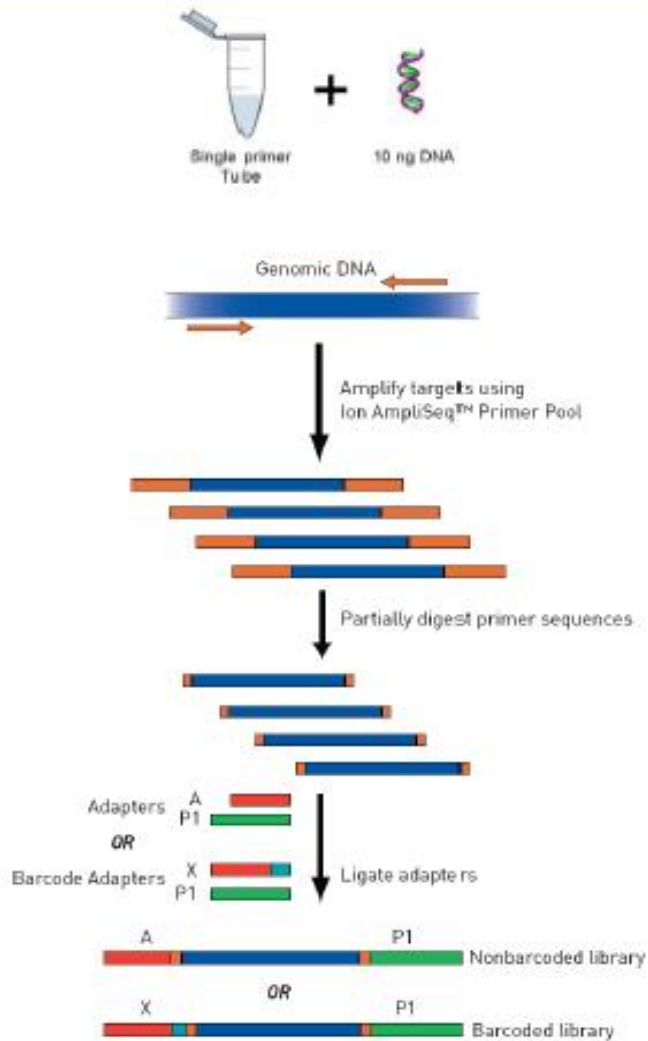


- Requires only 10 ng of FFPE or higher-quality gDNA, yields library in ~3.5 hours
- Panel targets >2,800 COSMIC mutations in 50 cancer-associated genes
- A single tube of primers for 207 amplicons (avg length= 154 bp)
- Samples can be barcoded and library-prep automated for multiplexing

## The 50 targeted genes

<i>ABL1</i>	<i>EZH2</i>	<i>JAK3</i>	<i>PTEN</i>
<i>AKT1</i>	<i>FBXW7</i>	<i>IDH2</i>	<i>PTPN11</i>
<i>ALK</i>	<i>FGFR1</i>	<i>KDR</i>	<i>RB1</i>
<i>APC</i>	<i>FGFR2</i>	<i>KIT</i>	<i>RET</i>
<i>ATM</i>	<i>FGFR3</i>	<i>KRAS</i>	<i>SMAD4</i>
<i>BRAF</i>	<i>FLT3</i>	<i>MET</i>	<i>SMARCB1</i>
<i>CDH1</i>	<i>GNA11</i>	<i>MLH1</i>	<i>SMO</i>
<i>CDKN2A</i>	<i>GNAS</i>	<i>MPL</i>	<i>SRC</i>
<i>CSF1R</i>	<i>GNAQ</i>	<i>NOTCH1</i>	<i>STK11</i>
<i>CTNNB1</i>	<i>HNF1A</i>	<i>NPM1</i>	<i>TP53</i>
<i>EGFR</i>	<i>HRAS</i>	<i>NRAS</i>	<i>VHL</i>
<i>ERBB2</i>	<i>IDH1</i>	<i>PDGFRA</i>	
<i>ERBB4</i>	<i>JAK2</i>	<i>PIK3CA</i>	

# Sample prep

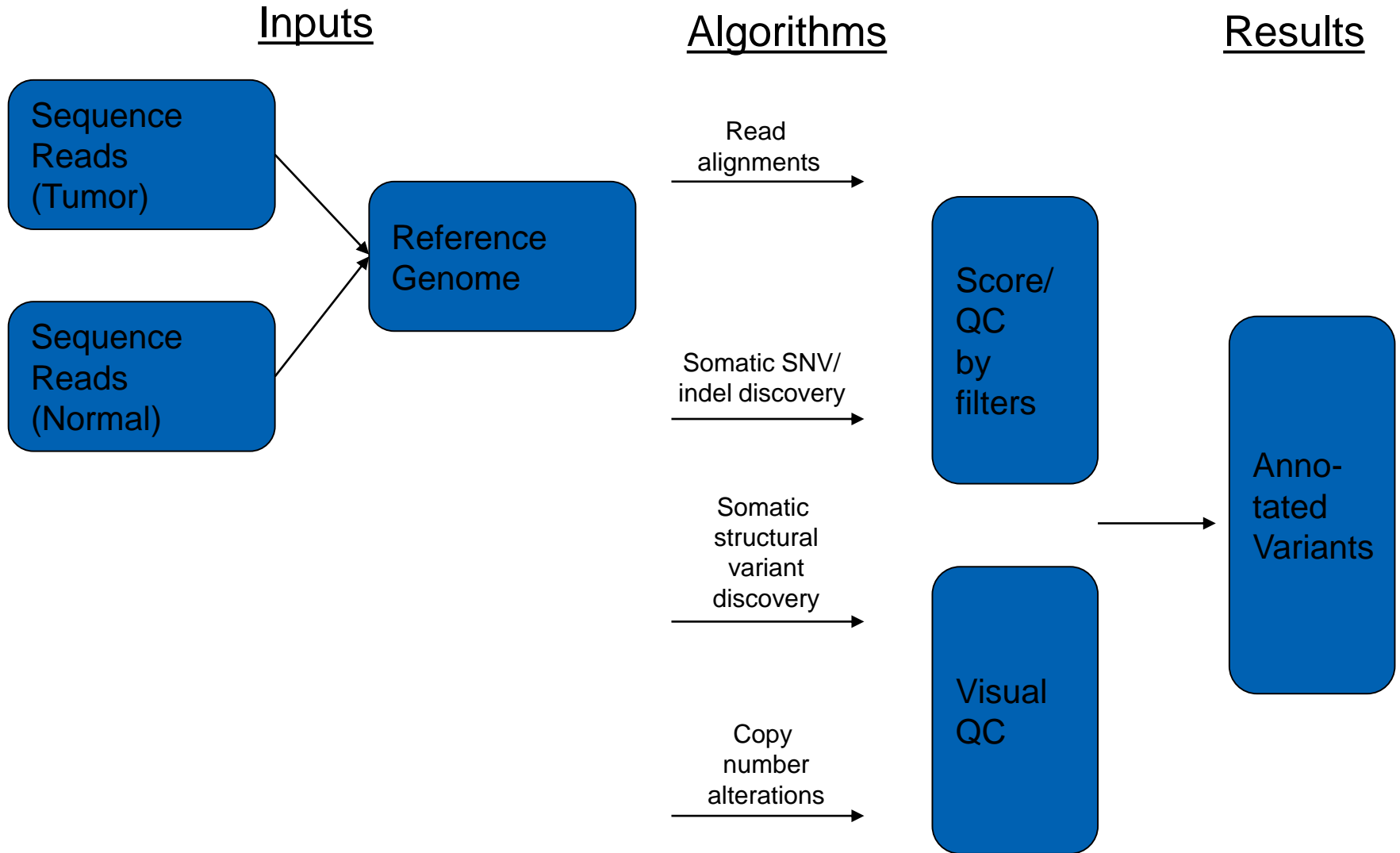


**Complete workflow for a single Ion 318™ Chip and 200 bp sequencing run**

CONSTRUCT LIBRARY	PREPARE TEMPLATE	RUN SEQUENCE	ANALYZE DATA	ANNOTATE RESULTS
<b>3.5</b> HOURS	<b>4</b> HOURS	<b>4.5</b> HOURS	<b>4</b> HOURS	<b>0.5</b> HOURS
ION AMPLISeq™ PANELS	ION TORRENT™ SYSTEM	ION PGM™ SEQUENCER	TORRENT SERVER	ION REPORTER™ SOFTWARE



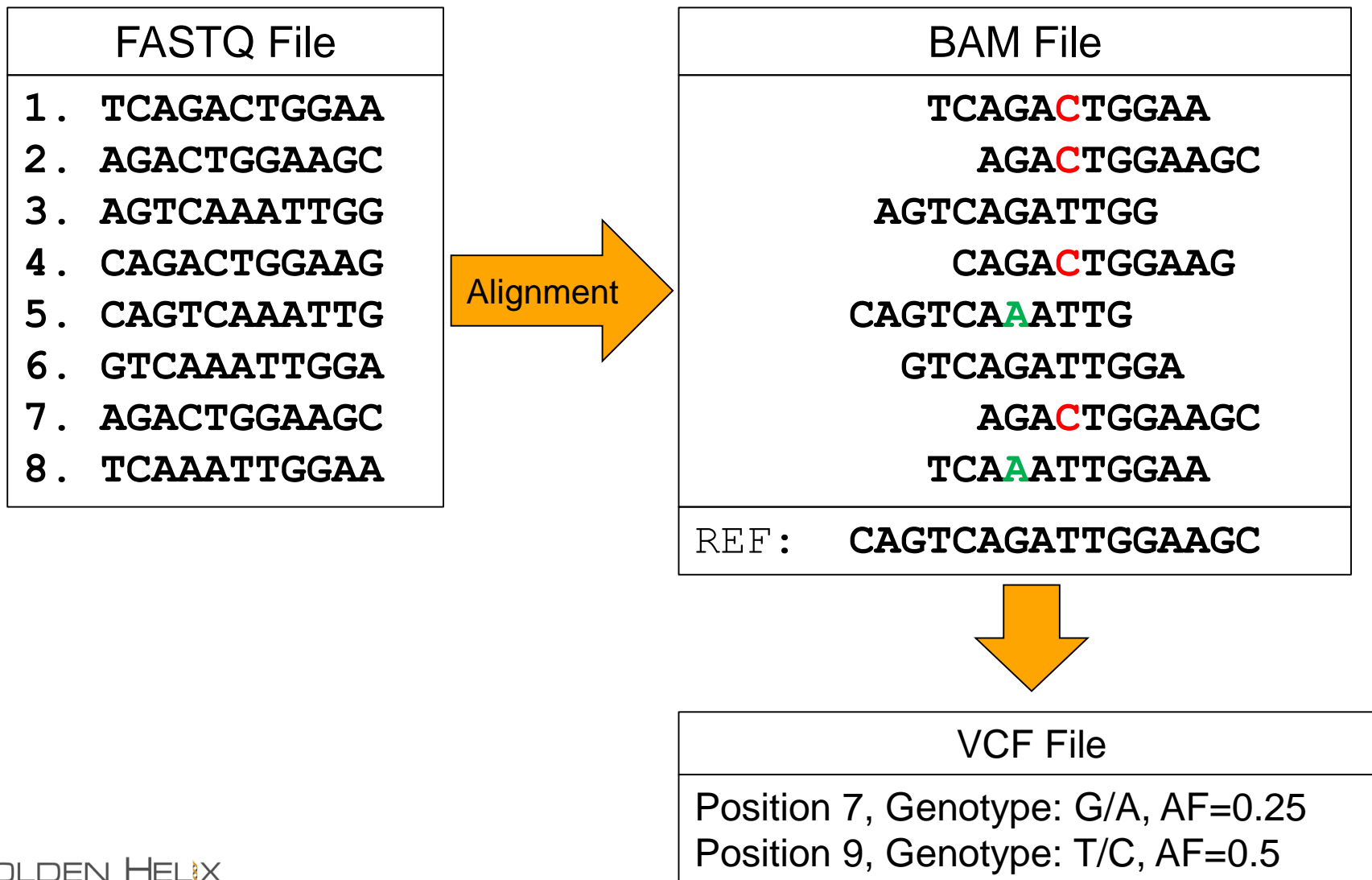
# Somatic Variant Discovery Pipeline



# Bioinformatics of Alignment



# Alignment and Variant Calling



# Calling a Variant in a tumor/normal sample



Project - Golden Helix GenomeBrowse 2.0.2

File View Tools Window Help

19 19: 36,045,890 - 36,045,915

Homo sapiens (Human), GRCh37 hg19 (Feb 2009)

Plot Tree

- 990005\_somatic\_hg19
- GastricCancerFeatures
- SRR504685.filtered.sorted.rea...
- Coverage
- Pile-up
- SRR504686.filtered.sorted.rea...

Console

History Copy Clear

Chr19: 36,045,903

Matches / Mismatches / Deletions

Type	Base	Count	% of Total	Mean Quality
(match)	T	230	76.4	29.4
(mismatch)	C	69	22.9	35.5
(mismatch)	G	2	0.7	5.5
Total		301	100	30.6

0 alignments filtered out by quality settings.

Chr19 between 36,045,903 and 36,045,904

Insertions

Base(s)	Count	% of Total	Mean Quality
Non-Insertions	306	100.0	?
Total	306	100	?

SRR504685.filtered.sorted.realigned.deduped.recalibrated RawData - GastricCancer

Coverage

Read Depth

A T G T A G A T G A G G T A G G G T G T C A G C T C

Pile-up

SRR504686.filtered.sorted.realigned.deduped.recalibrated RawData - GastricCancer

Coverage

Read Depth

A T G T A G A T G A G G T A G G G T G T C A G C T C

Pile-up

192

190

188

186

184

182

180

178

RefSeq Genes 63, UCSC

ATP4A Y I L Y P T L E

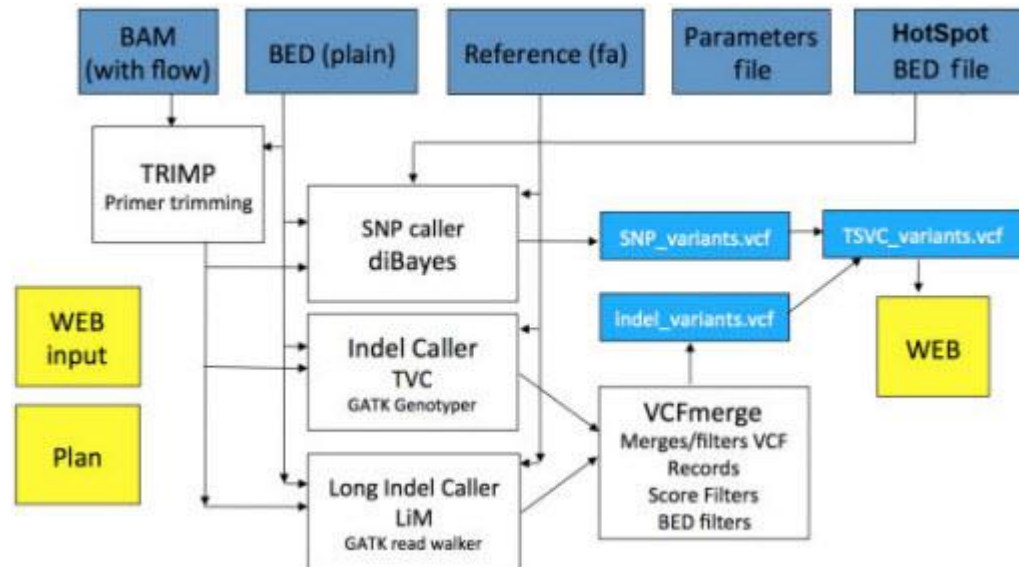
User Annotations

Point (19: 13,538,251, 557.723) 19 26 bp Update Available

# Torrent Server Variant Caller



- Configure parameters through Torrent Server Web interface
- TMAP Alignment Algorithm
- Custom Hybrid Variant Caller
- AmpliSeq Cancer Panel “Workflow”
  - Tuned to detect very low allele frequency variants
  - Input “HotSpot” file forces calls for known somatic variant sites via COSMIC
  - Target region BED provides coverage stats per region
  - Can customize thresholds for scenarios like circulating tumor cells



File Tools Help

Import Export ?

Cancer Panel Workflow (2 Variants) Cancer Panel Workflow (2 Gene Names) Cancer Panel Workflow

E107279-058b03-12-L7555

Variant Sites Annot... Summary of COSMIC Mutations Left Aligned 71, GHI

Chr:Pos	Ref/Alt	Gene Names	Alt Allele Freq	In COSMIC?	Mutation ID (Unique)	Mutation CDS (U...	Mutation AA (U...
2:209113192	G/A	IDH1	0.0395792	True	1741220	c.315C>T	p.G105G
3:178927410	A/G	PIK3CA	0.0600601	True	328028	c.1173A>G	p.I391M

Filter 1,685

Read Depths (DP) (Current 1,500)

- Less than 1,500: 181
- Equal to 1,500: 0
- Greater than 1,500: 1,504
- Missing: 0

Alt Allele Freq (Current 0.01)

- Less than 0.01: 1,492
- Equal to 0.01: 0
- Between 0.01 and 0.3: 5
- Equal to 0.3: 0

In COSMIC? True: 2

Detail GenomeBrowse

History

Sample Fields Table

Samples	Genotypes	Genotype Qualities (GQ)	Alt Allele Freq
E107279-058b03-12-L7555	A_G	99	0.0600601

Annotate Transcripts


Gene Names: PIK3CA

Summary of COSMIC Mutations Left Aligned 71, GHI

In COSMIC?	True
Mutation ID (Unique)	328028
Mutation CDS (Unique)	c.1173A>G
Mutation AA (Unique)	p.I391M

Variant+Transcript Interactions

Transcript Name	Sequence Ontology	HGVS c.	HGVS p.	Effect
1 NM_006218.2	missense_variant	NM_006218.2:c.1173A>G	NP_006209.2:p.Ile391Met	Missense



Point (3: 106,726,375, 0.9625) 3 20 bp

# Annotation Sources



COSMIC: Catalogue Of Somatic Mutations In Cancer

[cancer.sanger.ac.uk/cancergenome/projects/cosmic/](http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/)

**COSMIC**  
Catalogue of somatic mutations in cancer

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Search COSMIC v71

Search Gene name, Mutation, Tissue, Sample ...

By Cancer  
By Gene  
By Sample

eg. BRAF, V600E, lung, COLO-829

Search via [Cancer Browser](#)

**COSMIC**

All cancers arise as a result of the acquisition of a series of fixed DNA sequence abnormalities, mutations, many of which ultimately confer a growth advantage upon... [\[More\]](#)

**Cosmic Release v71**

COSMIC v71 includes full literature curation of PTNRB, PTCG1, POT1 and STAG2, the addition of 25 new census genes and an update of gene expression and copy number data from ICGC release 17 (Sept 2014)... [\[More\]](#)

Statistics	
Genes	28977
Samples	1058292
Coding Mutations	2710449
Papers	20247
Unique Variants	2139424
Fusions	10567
Genomic Rearrangements	61232
Whole Genomes	15047
Copy Number	702652
Gene Expression	118886698

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**Cancers Selected for Study**

The Cancer Genome Atlas researchers are mapping the genetic changes in 20 cancers. Find out which cancers have been selected for study, the criteria for selection and the scientific questions being asked about each cancer. [Learn More](#)

Launch Data Portal

Questions About Cancer

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<https://clinicaltrials.gov>

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ClinicalTrials.gov is a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world. [Learn more about clinical studies and about this site, including relevant history, policies, and laws.](#)

Now Available for Public Comment: Notice of Proposed Rulemaking (NPRM) for FDAAA 801 and NIH Draft Reporting Policy for NIH-Funded Trials

Find Studies About Clinical Studies Submit Studies Resources About This Site

ClinicalTrials.gov currently lists 182,594 studies with locations in all 50 states and in 187 countries. Text Size

Search for Studies  
Example: "Heart attack" AND "Los Angeles"

Advanced Search | See Studies by Topic | See Studies on a Map

Search Help

- How to search
- How to find results of studies
- How to read a study record

Locations of Recruiting Studies

Total N = 34,362 studies  
Data as of January 20, 2015

- Non-U.S. Only (52%)
- U.S. Only (42%)
- Both U.S. and Non-U.S. (6%)

See more trends, charts, and maps

[www.mycancergenome.org](http://www.mycancergenome.org)

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Home DIRECT About Us

**Find a Cancer Mutation**

Disease (required): Select Disease

Gene (optional): Select Disease First

Variant (optional): Select Disease First

GO

**Find Clinical Trials**

Lists trials by Disease or Gene for all national and international trials registered within [PDQ](#) and [clinicaltrials.gov](#).

Disease (optional): Enter a Disease

Gene (optional): Enter a Gene

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# VarSeq DEMO







- Extensive expert curated cancer focused knowledge base
- OncoMD
  - Variant level annotations
  - Gene information
  - Drug information
    - On label use
    - Off label use
    - Response rate
    - Clinical trial information
- Integration with VarSeq coming soon

Gene	Function	Alterations	Pathways	Frequency In Patient's cancer (%)	Approved Drugs	Mutations sensitive to drugs	Mutations resistant to drugs
KIT	Oncogene	Mutation	Angiogenesis	1.9	NA	NA	NA
BRAF	Oncogene	Mutation	MAPK signalling	0.6	NA	NA	NA
PIK3CA	Oncogene	Mutation	PI(3)K signalling	0.6	NA	NA	NA
PIK3CA	Oncogene	Amplification	PI(3)K signalling	23-40	NA	NA	NA

3) Background information of the gene

#	Gene	Background
1	KIT	The KIT gene encodes a transmembrane receptor kinase (RTK) protein and somatic activating mutations in KIT have been associated with a majority of Gastro-Intestinal Stromal Tumors (GIST). The majority of these mutations (67%) are reported in exon 11 followed by about 11% in exon 9. Rarely (1.5%-4.5%) mutations are reported in exons 13 or 17 too although the frequency of these are reported to be lower in
2	BRAF	
3	PIK3CA	

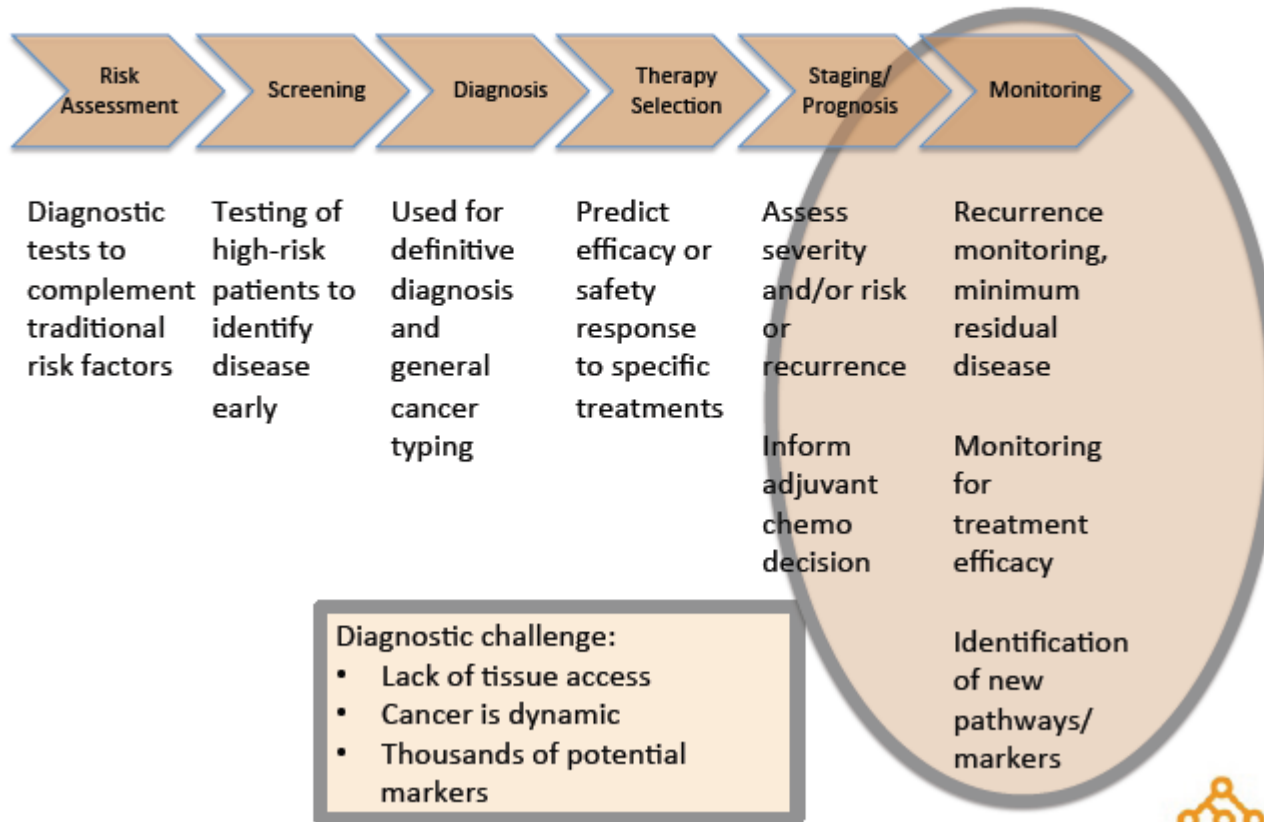



1) Variant details

#	Gene	CDS Change	Amino acid change	Approved drug	Approved in patient's cancer	Approved in other cancer	Response rate (%)	Number of Samples
1	BRAF	c.1799T>A	p.V600E	Dabrafenib + Trametinib(Tafinlar + Mekinist)	NA	Melanoma, Skin cancer, meningioma	NA	15
2	BRAF	c.1799T>A	p.V600E	Dabrafenib(Tafinlar)	NA	Colorectal cancer, Gastrointestinal cancer, Leukemia, Melanoma, Skin cancer, Thyroid cancer, meningioma	NA	33
3	BRAF	c.1799T>A	p.V600E	Sorafenib(Nexavar)	NA	Melanoma, Thyroid cancer	NA	22
4	BRAF	c.1799T>A	p.V600E	Sunitinib(Sutent)	NA	Skin cancer, Thyroid cancer	NA	4
5	BRAF	c.1799T>A	p.V600E	Trametinib(Mekinist)	NA	Melanoma	NA	3
6	BRAF	c.1799T>A	p.V600E	Vemurafenib(Zelboraf)	NA	Brain cancer, Leukemia, Lung cancer, Melanoma, Myeloma, Skin cancer, Thyroid cancer	NA	385



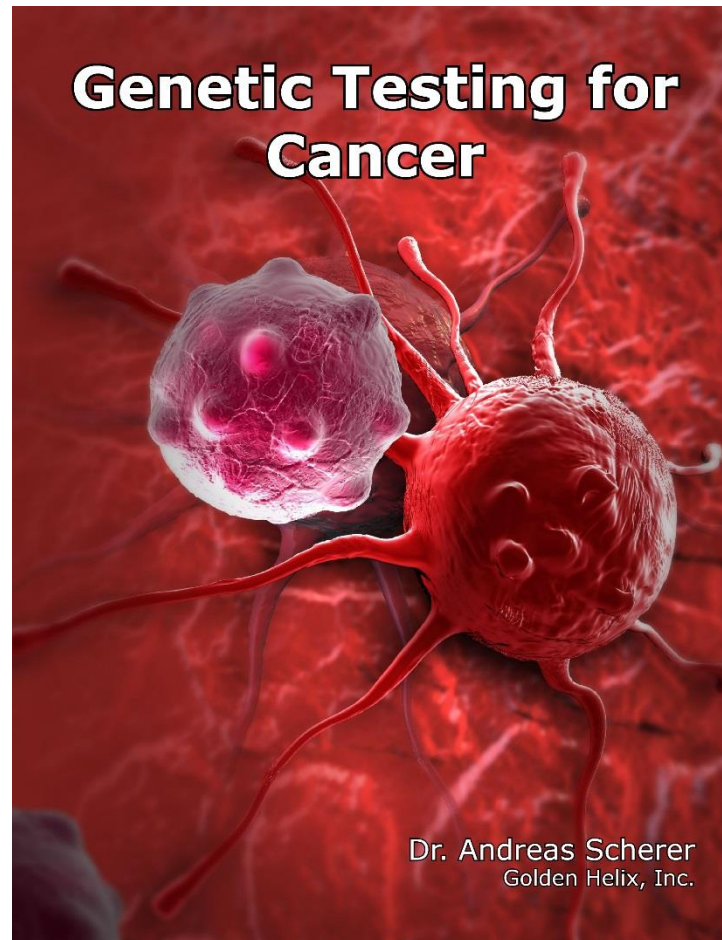
## The biggest unmet need is monitoring



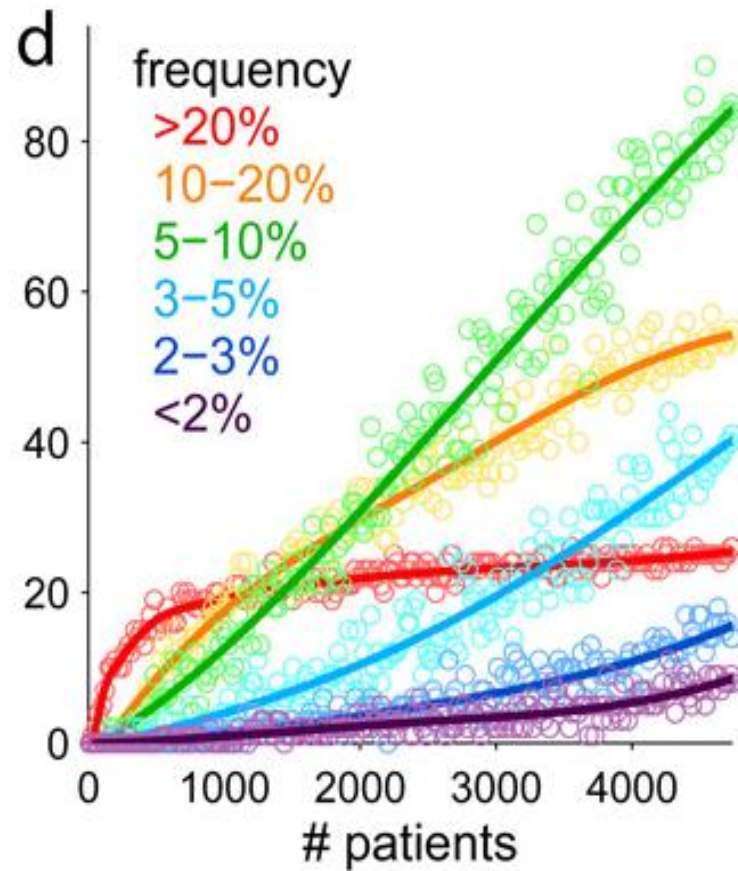
# Introductory period for VarSeq ends March 31 2015



- **Launch in October 2014 covered by GenomeWeb. Just released VarSeq 1.1.1: Gene ranking via PhoRank, Custom Variant Data Base, Log File (Compliance), Note Editor (Reporting)**
- **Presentations at ASHG in October 2014 and TriCon 2015**
- **Customer Announcements**
  - Northshore Next Gen Sequencing Lab (see press release)
  - Prevention Genetics (see <https://www.genomeweb.com/business-news/preventiongenetics-use-goldenhelix-varseq-dx-test-offering>)
- **Partnerships**
  - Fluxion Bio
  - MedGenome



<http://goldenhelix.com/resources/ebooks/GeneticTestingforCancer.html>



Lawrence et al, Nature 2014



# Questions?

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