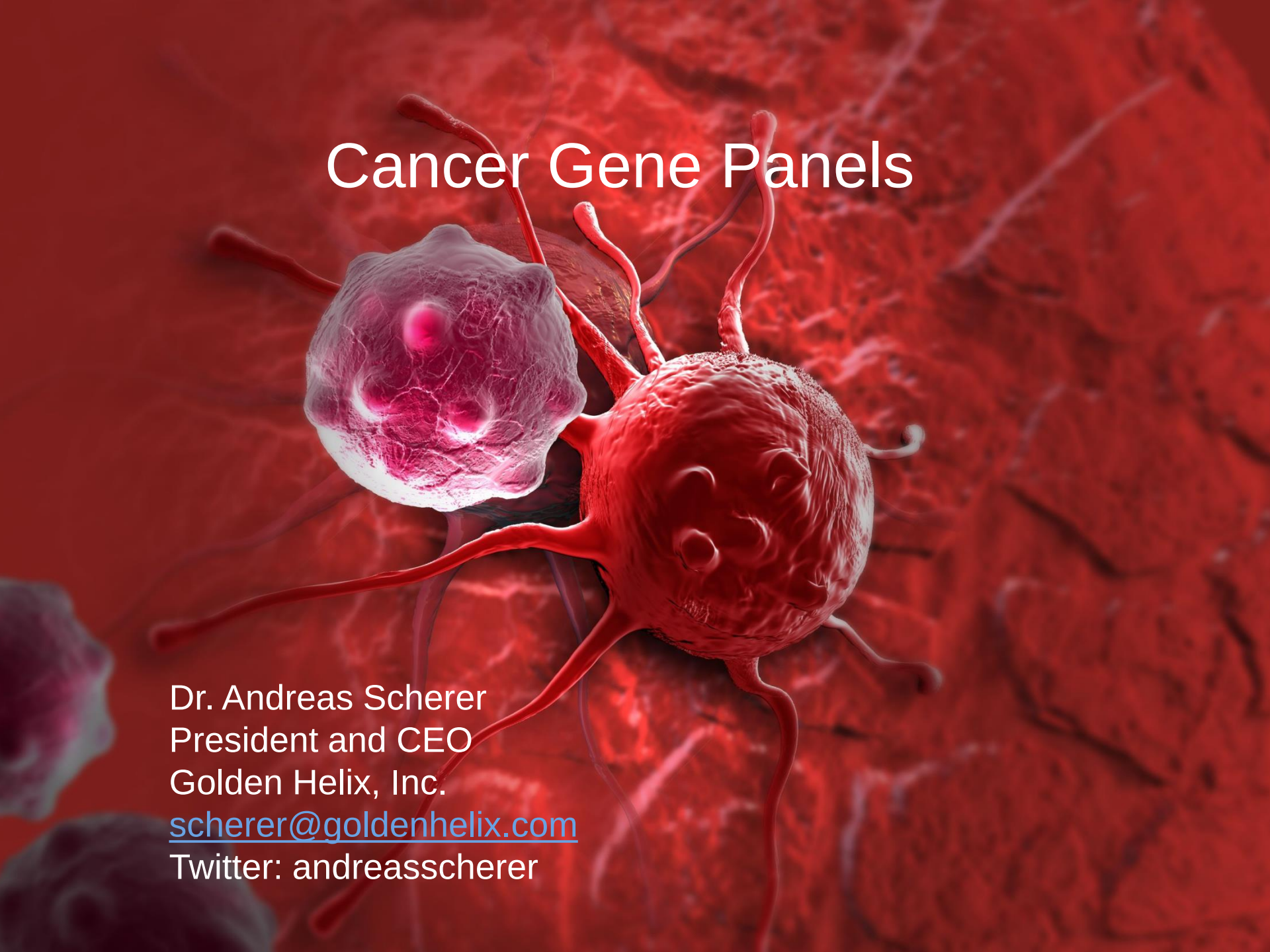
Cancer Gene Panels



Dr. Andreas Scherer
President and CEO
Golden Helix, Inc.
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About Golden Helix

- 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

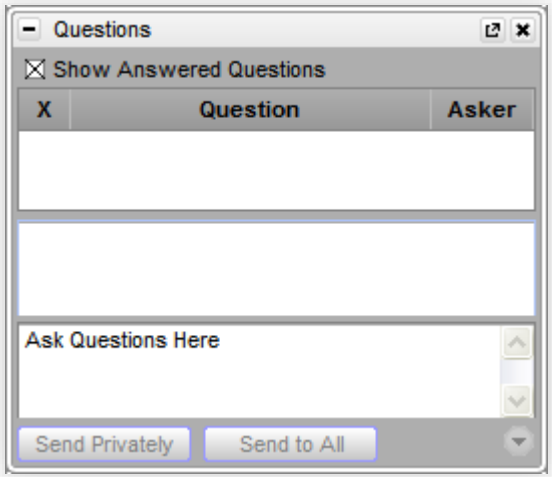
Cancer is a disease of the genome!

Theodore Boveri (1862 – 1915)

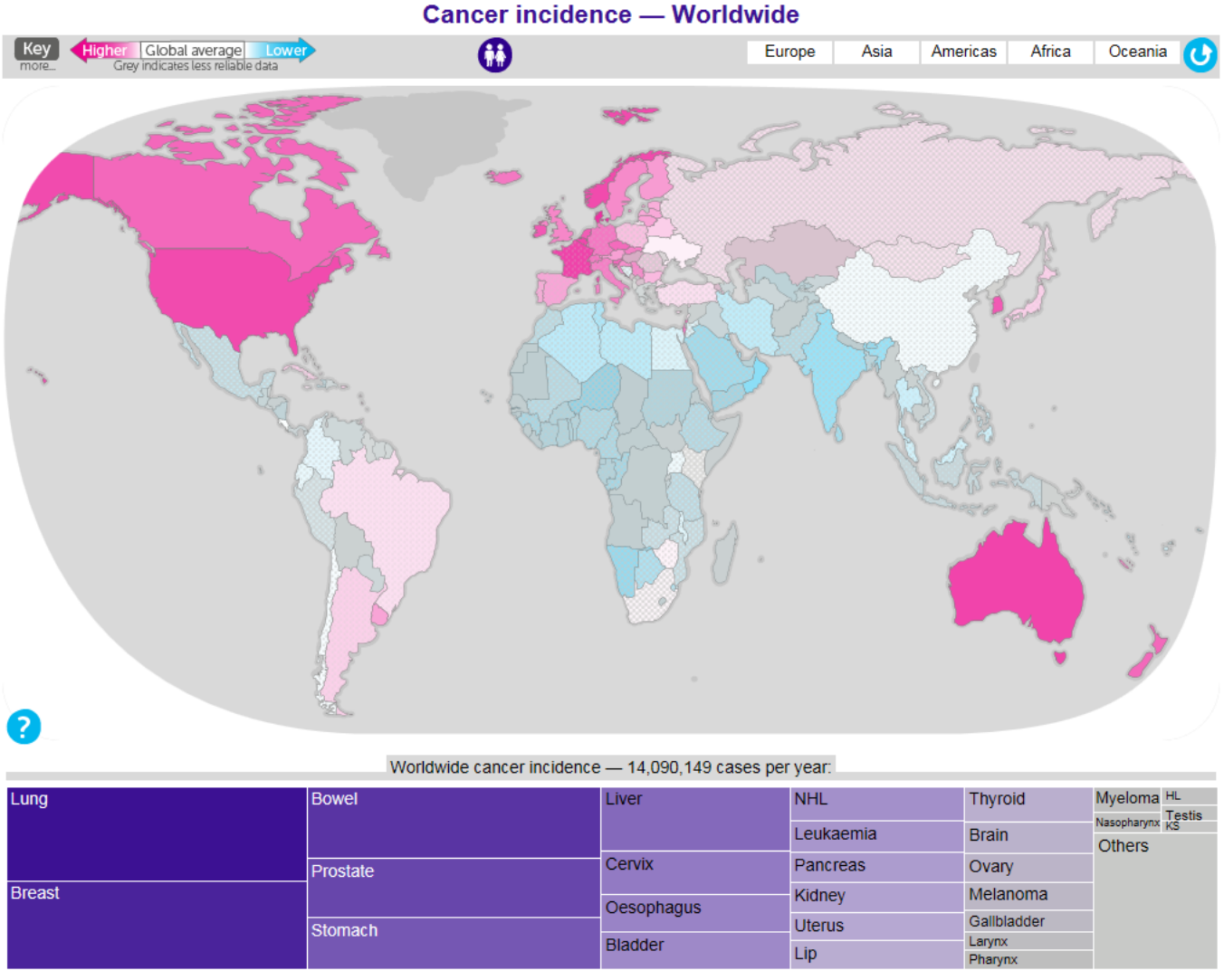
Sea Urchins

Questions during the presentation

Use the Questions pane in your GoToWebinar window

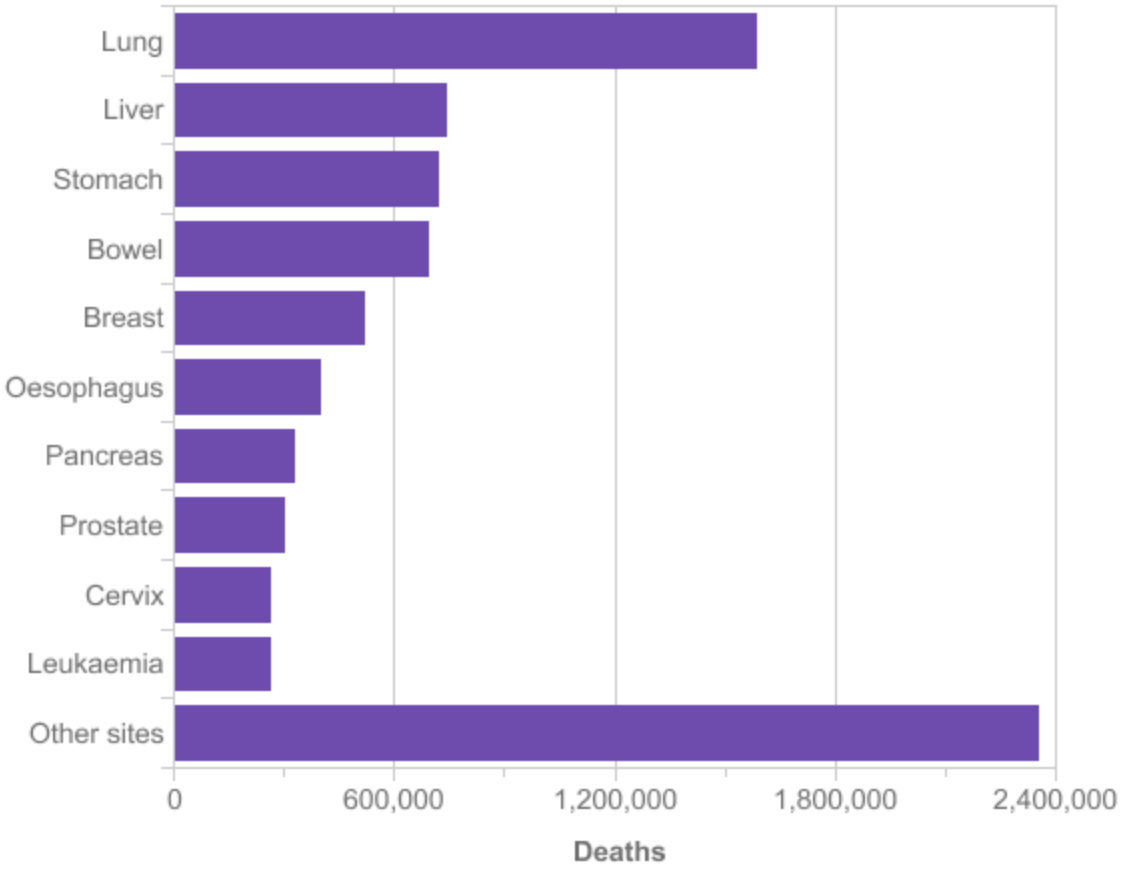


Global cancer incidence



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

Global mortality figures



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

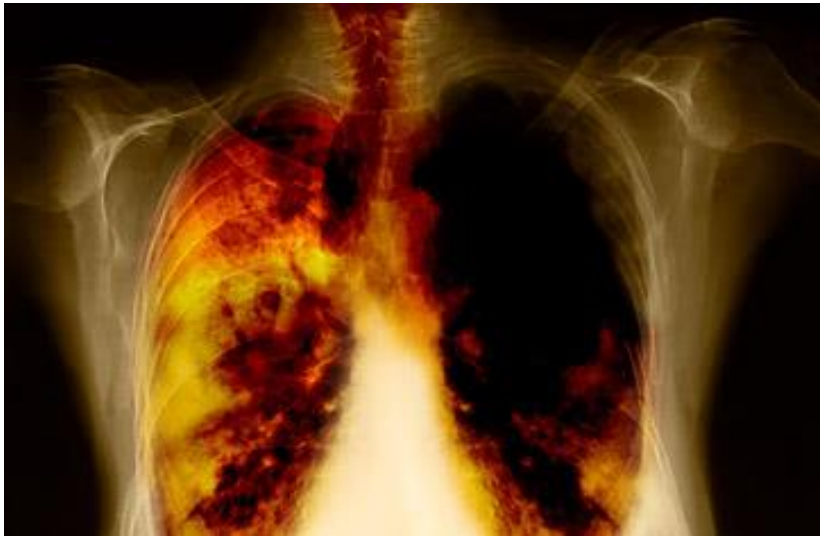
Global numbers

- **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.**

Lung Cancer



- 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%.

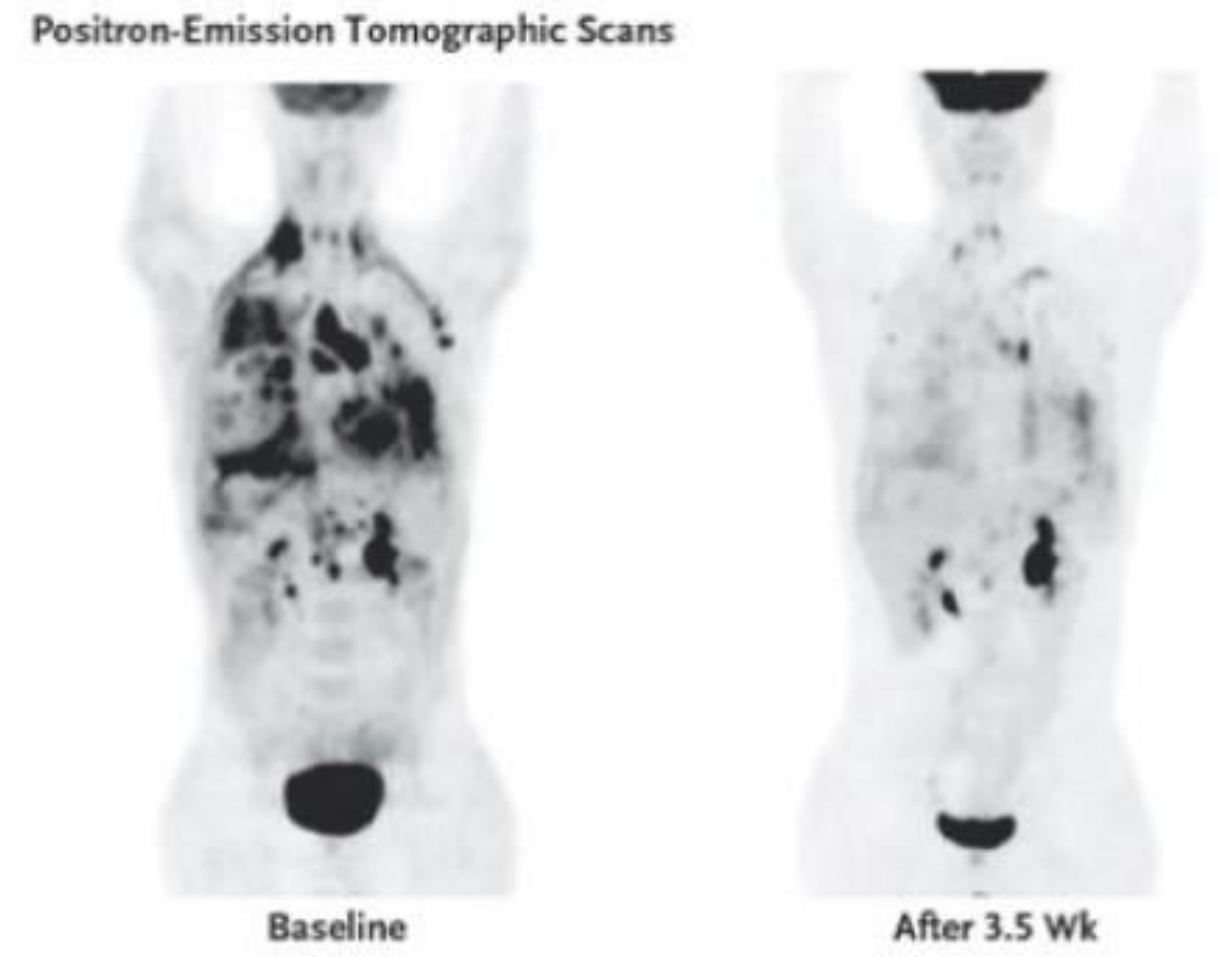
Lung Cancer

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

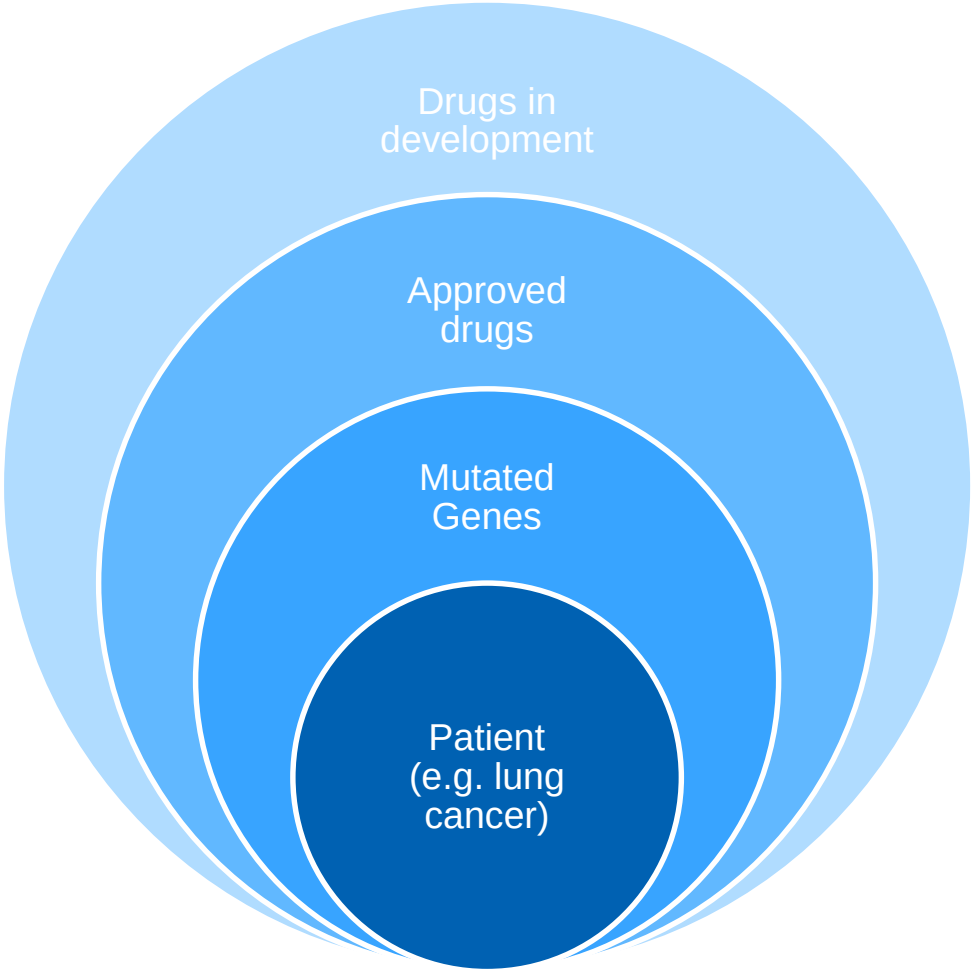


Cost of testing in personalized medicine

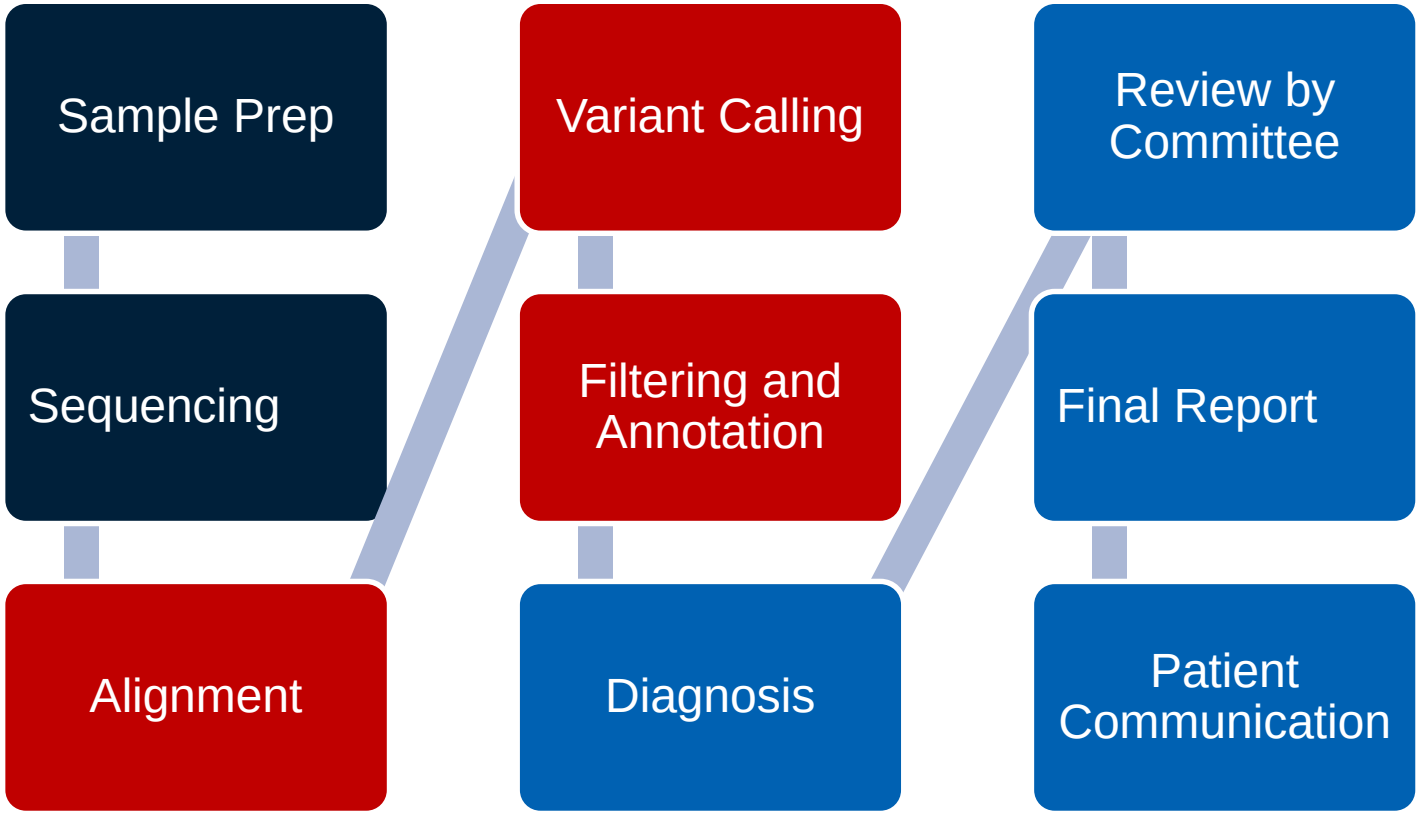
- Result of recent phase III study compared crizotinib with standard chemotherapy in patients with locally advanced or metastatic *ALK*-positive lung cancer:
  - median progression-free survival (PFS), 7.7 versus 3.0 months;
  - response rate: 65% versus 20%; and
  - symptoms and quality of life were also substantially better.
  - 64% of the group receiving chemotherapy crossed over to crizotinib.
- Besides the cost of the drug itself, one main questions 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-Positve, 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



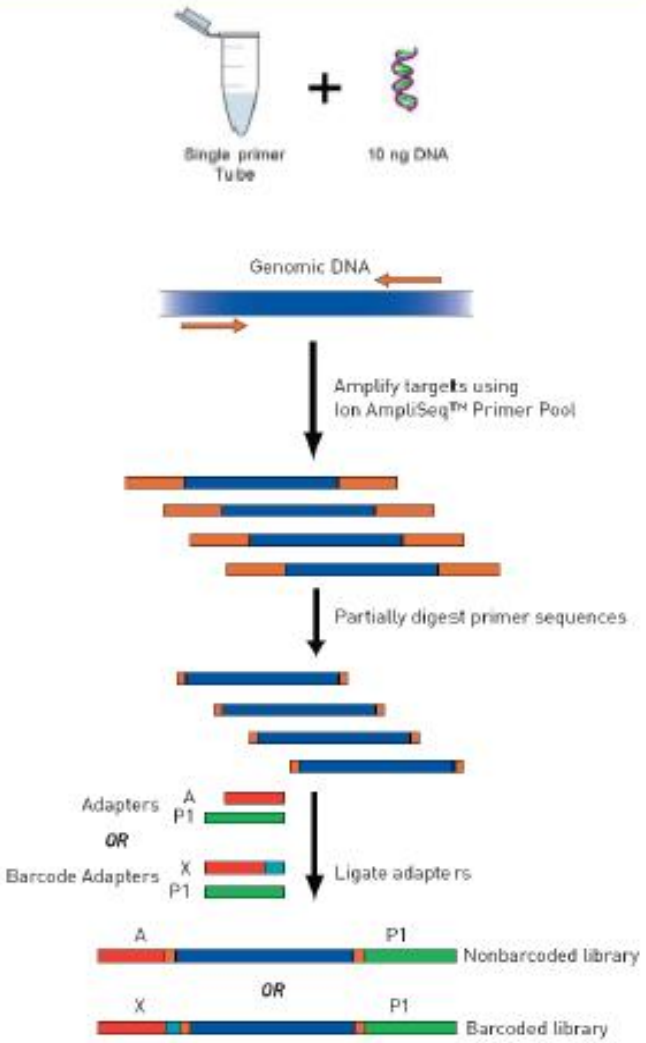
Ion AmpliSeq Cancer Hotspot Panel

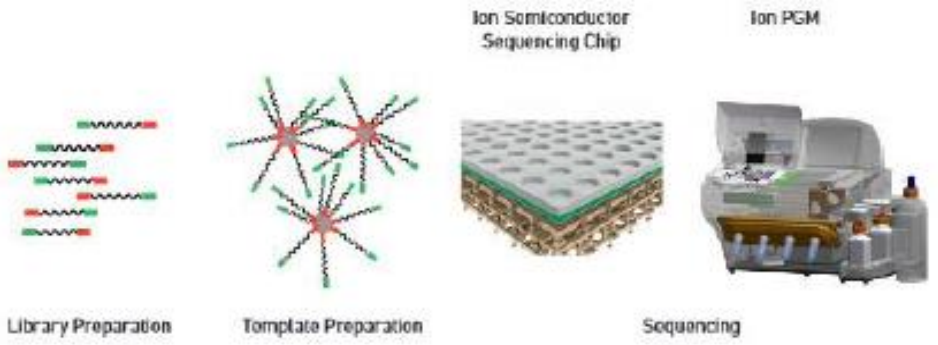
- Requires only 10 ng of FFPE or higher-quality gDNA, yields library in ~3.5 hours
- Panel targets >2,800 COSMIC mutations in 50 cancer-associated genes
- A single tube of primers for 207 amplicons (avg length= 154 bp)
- Samples can be barcoded and library-prep automated for multiplexing

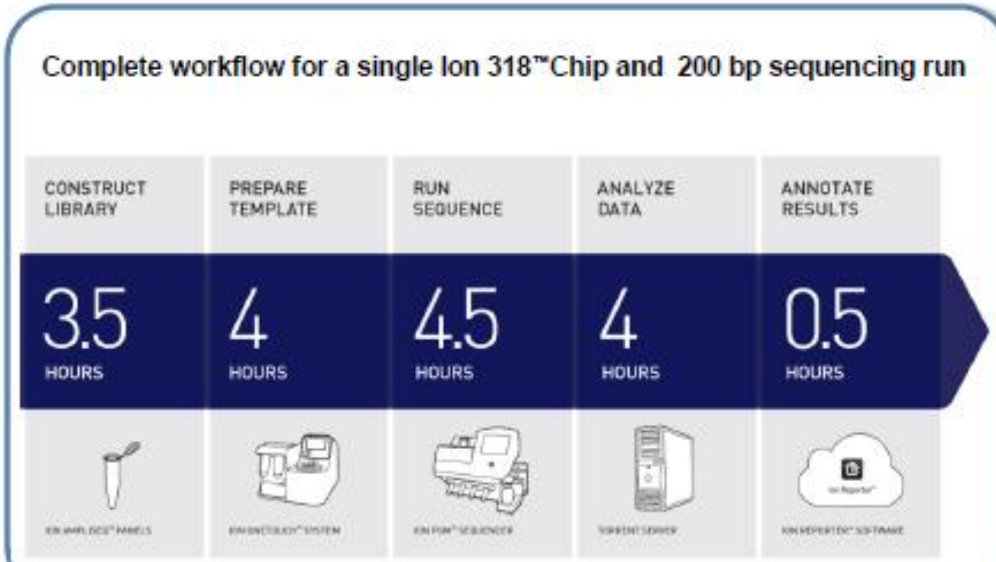
The 50 targeted genes

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

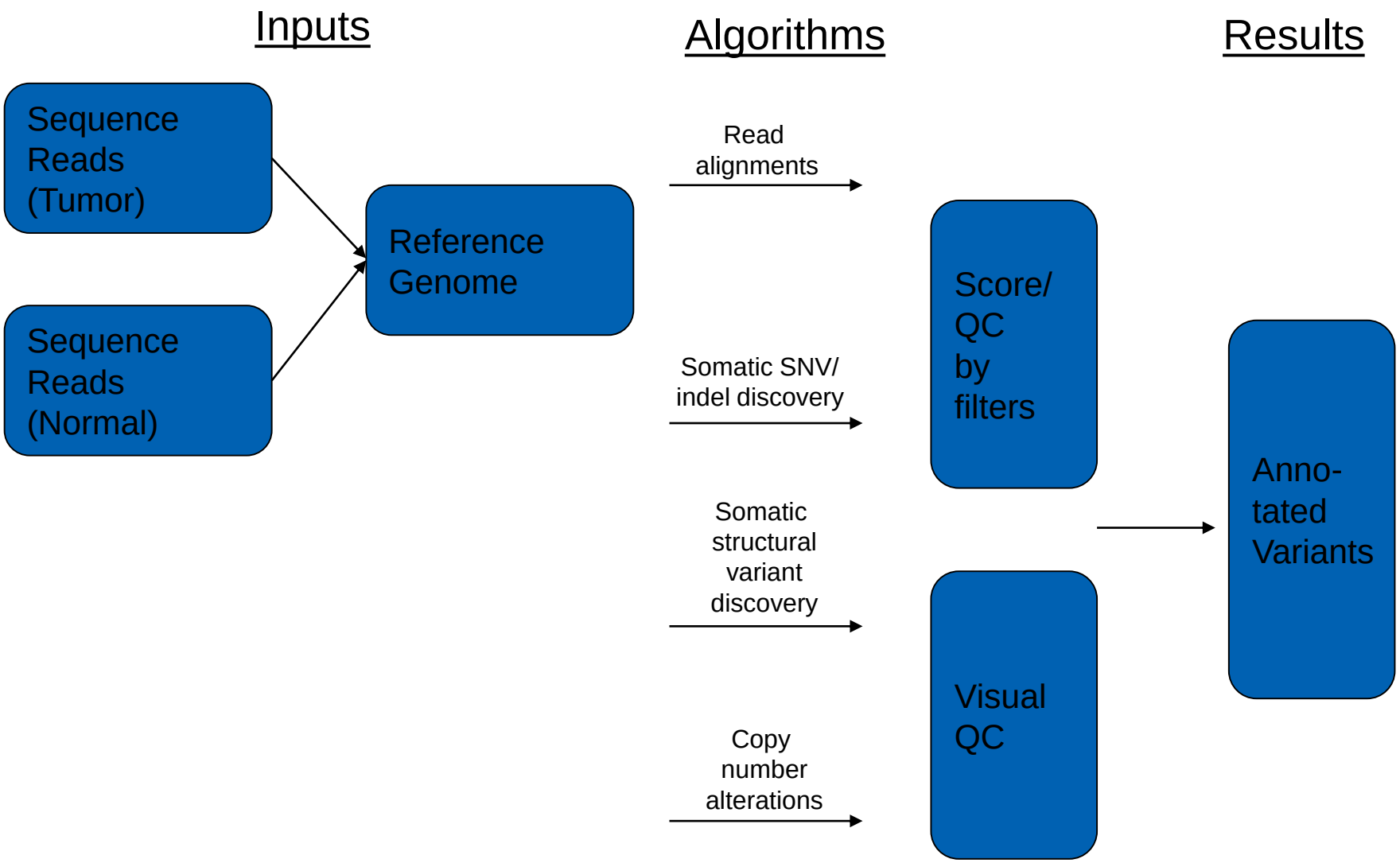
Sample prep


Single primer Tube
10 ng DNA
Genomic DNA
Amplify targets using Ion AmpliSeq™ Primer Pool
Partially digest primer sequences
Adapters
A
P1
OR
Barcode Adapters
X
P1
Ligate adapters
A
P1
Nonbarcoded library
OR
X
P1
Barcoded library


Ion Semiconductor Sequencing Chip
Ion PGM
Library Preparation
Template Preparation
Sequencing

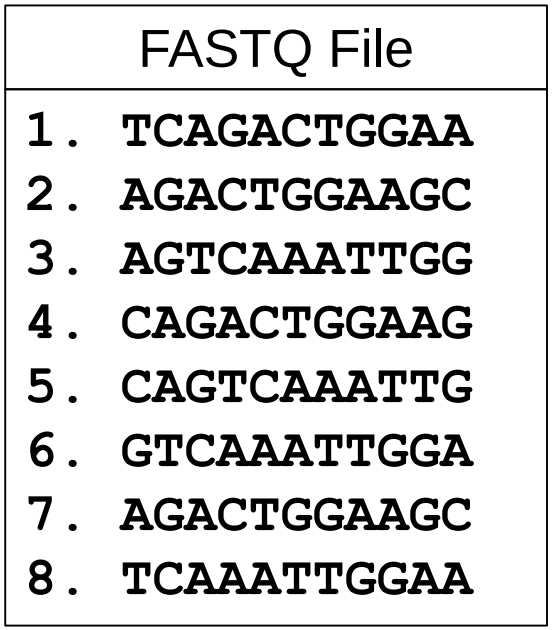

Complete workflow for a single Ion 318™Chip and 200 bp sequencing run
CONSTRUCT LIBRARY
PREPARE TEMPLATE
RUN SEQUENCE
ANALYZE DATA
ANNOTATE RESULTS
3.5 HOURS
4 HOURS
4.5 HOURS
4 HOURS
0.5 HOURS

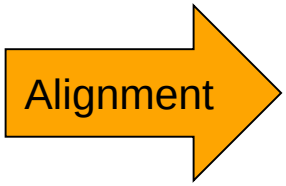
Somatic Variant Discovery Pipeline

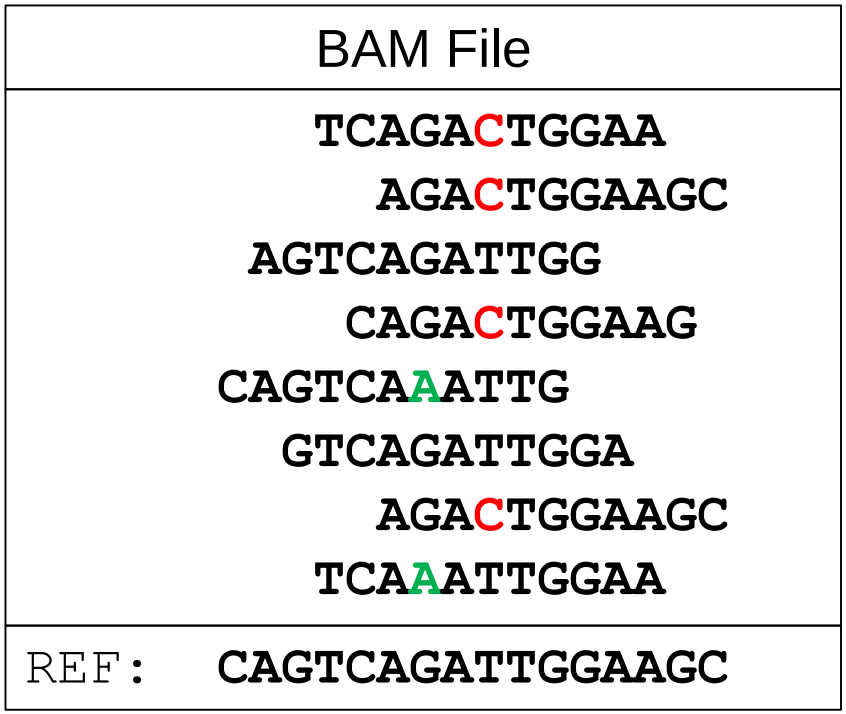


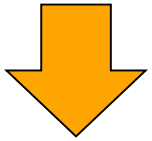
Bioinformatics of Alignment

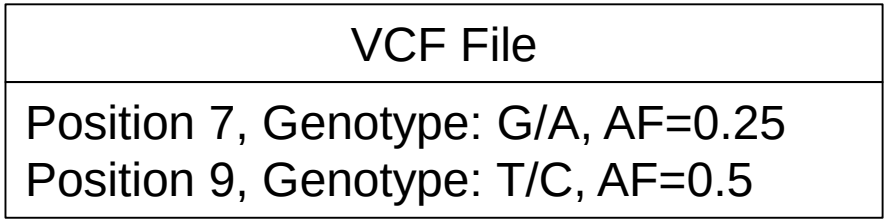
Alignment and Variant Calling



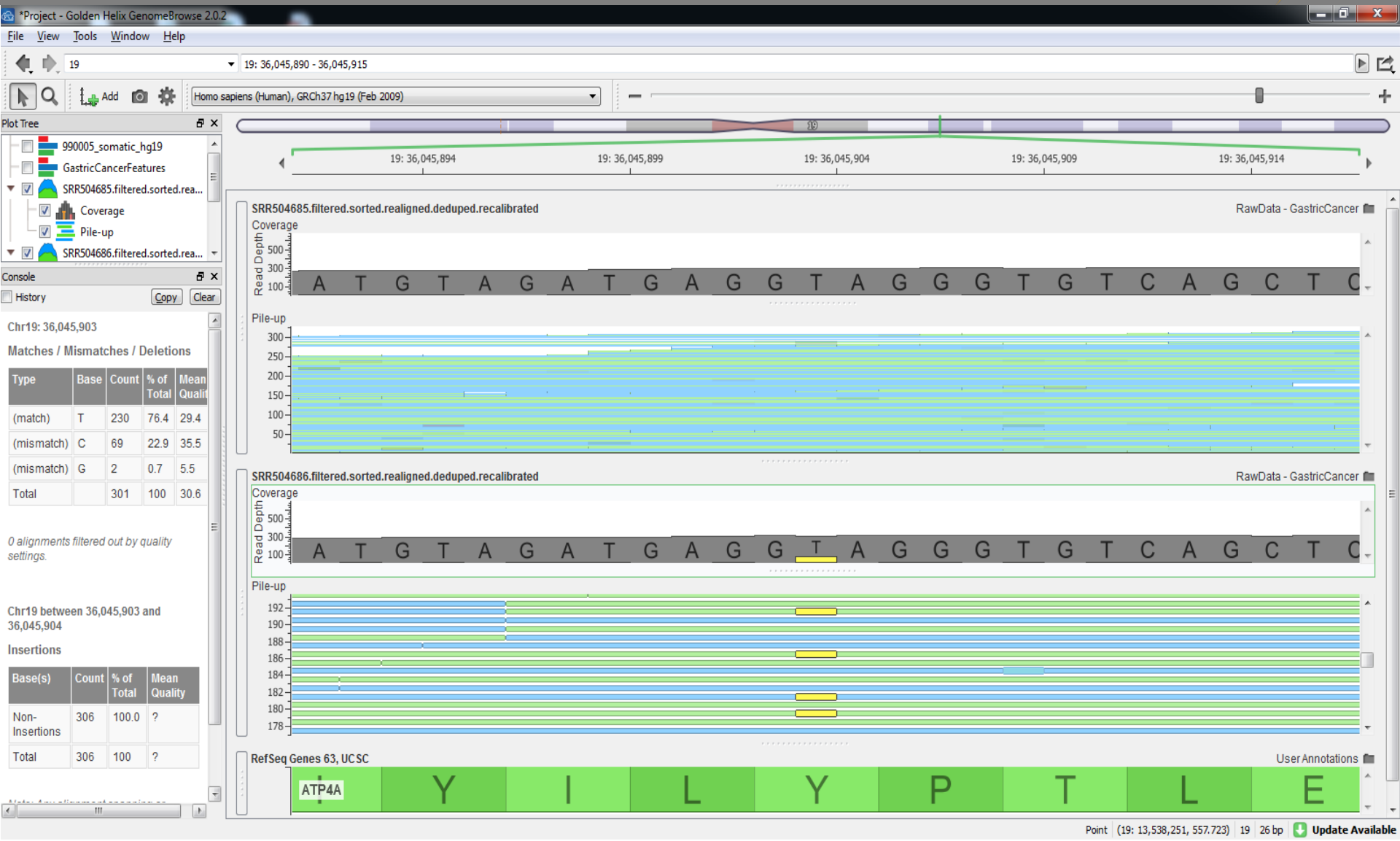






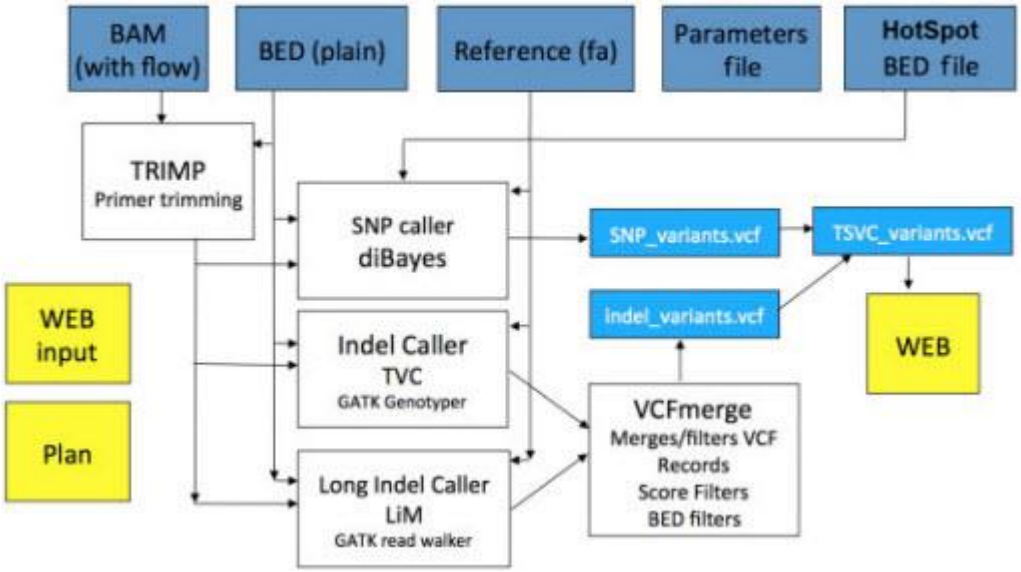


Calling a Variant in a tumor/normal sample



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 freq 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



Ion Torrent CHPv2 Example - Golden Helix VarSeq 1.1.0

File Tools Help

Import Export

Cancer Panel Workflow

E107279-058b03-12-L7555

Cancer Panel Workflow 1,708

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
	1,504

Alt Allele Freq (Current)

0.01

0.3

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

In COSMIC?

True	2
False	3
Missing	0
	2

2

(2 Variants) Cancer Panel Workflow

(2 Gene Names) Cancer Panel Workflow

Add Export Cancer Panel Workflow Variants: 2

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

Detail GenomeBrowse

History Copy Clear

Sample Fields Table

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

Show 16 hidden fields

Annotate Transcripts

Gene Names	PIK3CA

Show 6 hidden fields

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

Show 4 hidden fields

Variant+Transcript Interactions

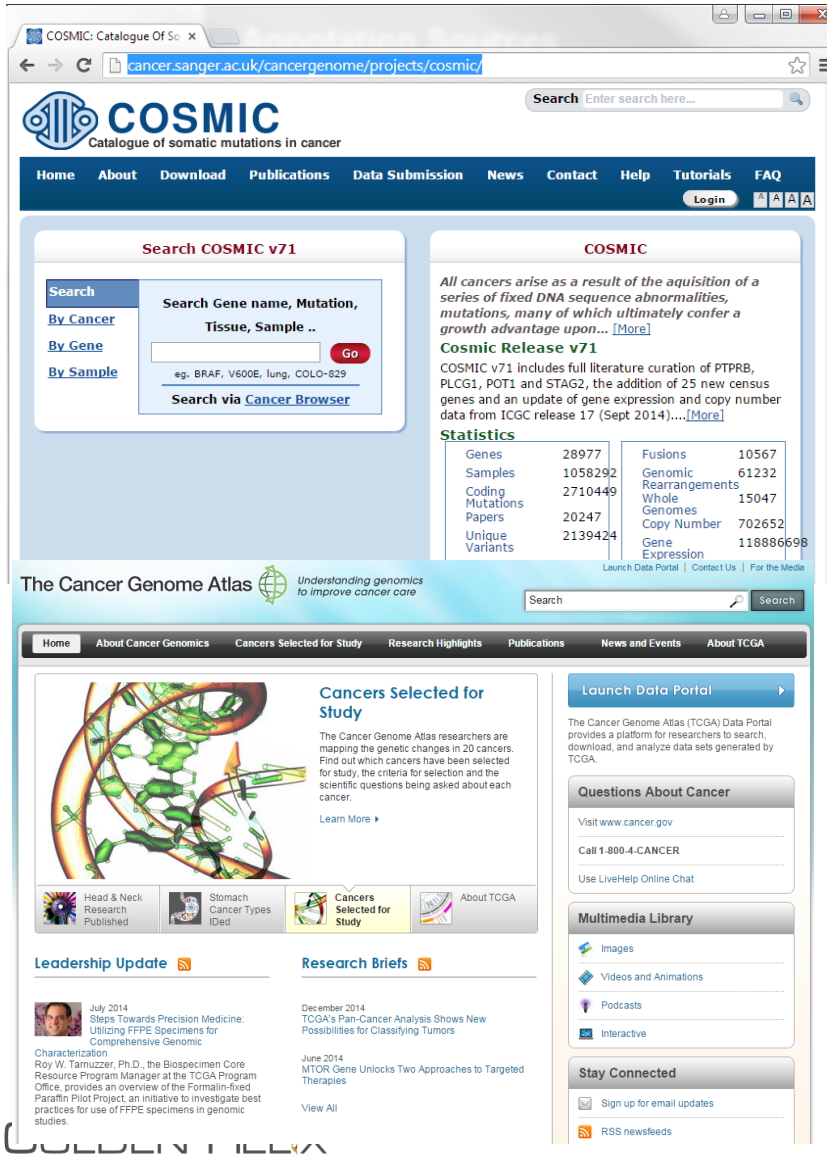
	Transcript Name	Sequence Ontology	HGVS c.	HGVS p.	Effect
1	NM_006218.2	missense_variant	NM_006218.2:c.1173A>G	NP_006209.2:p.Ile391Met	Missense

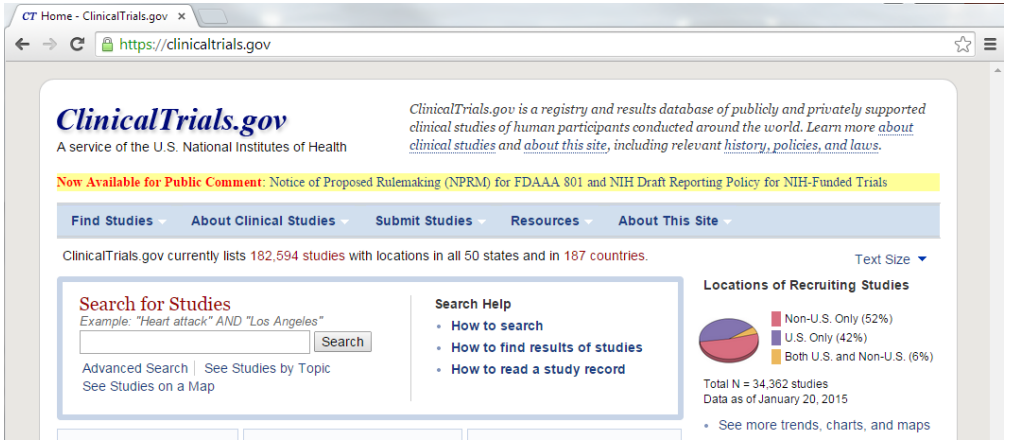
Show 9 hidden fields

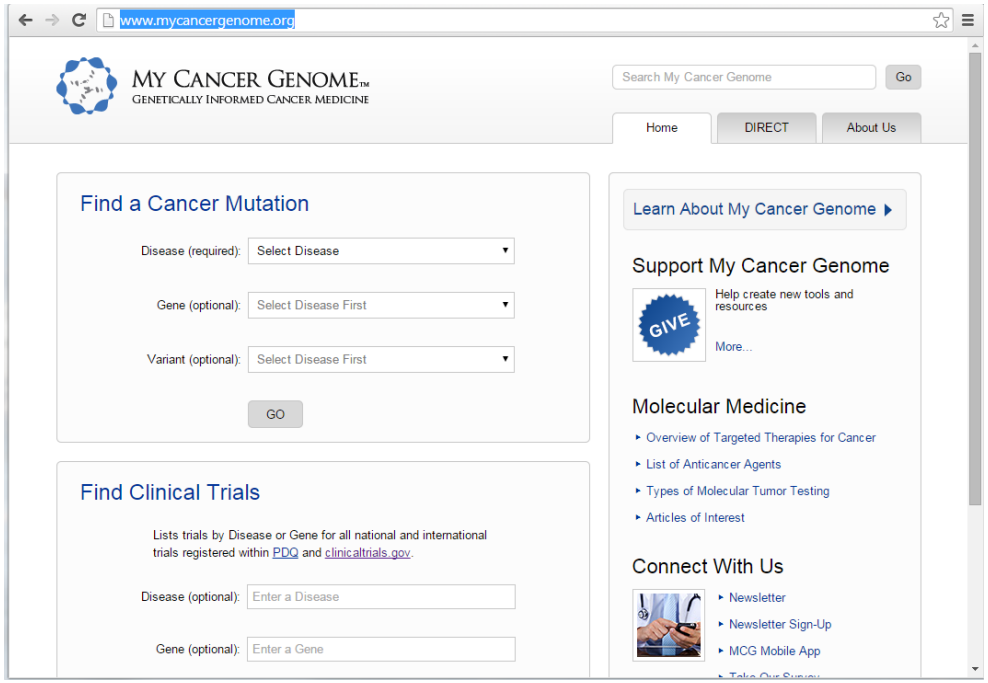
varSEQ™

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

Annotation Sources



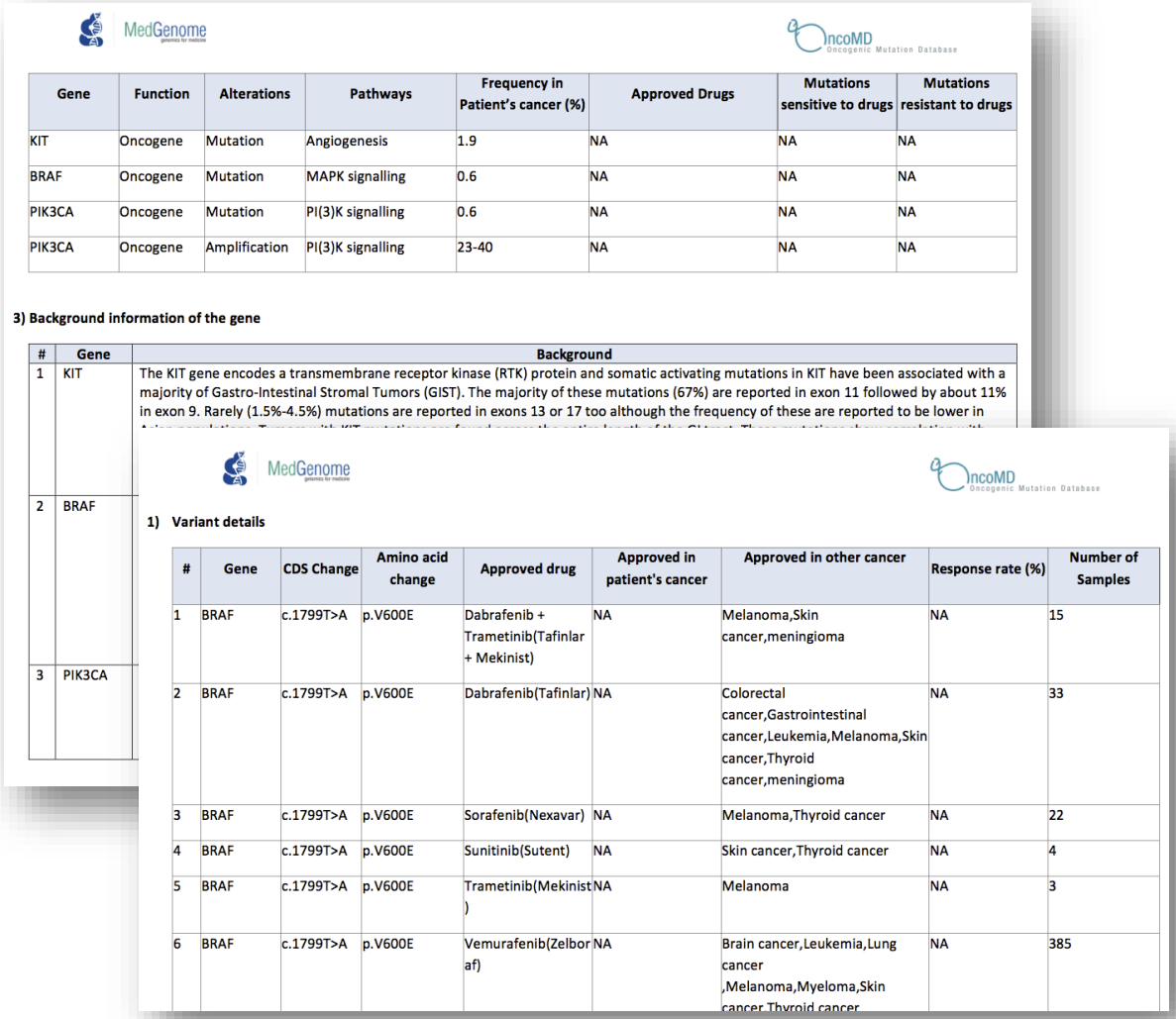




VarSeq DEMO

MedGenome Partnership

- **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**



MedGenome

OncoMD Oncogenic Mutation Database

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	

MedGenome

OncoMD Oncogenic Mutation Database

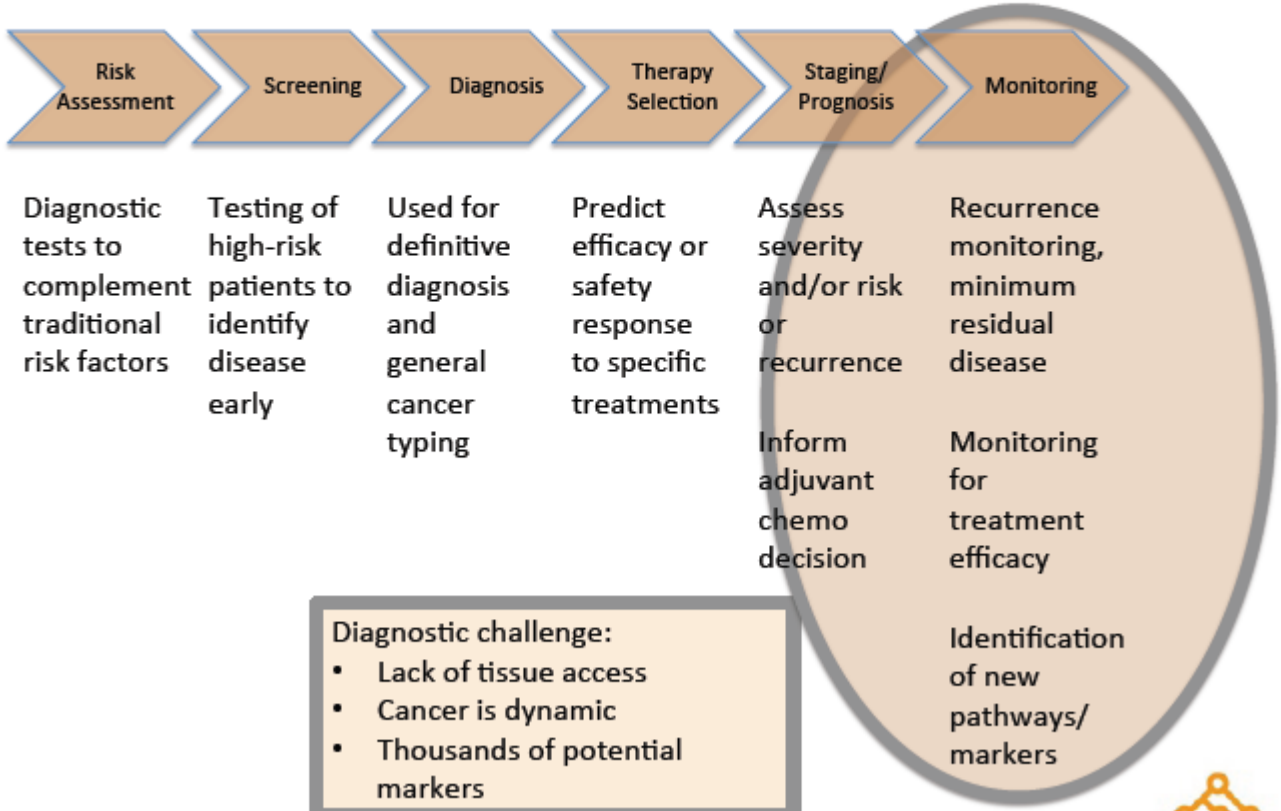
1) Variant details

#	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

GOLDEN HELIX
Accelerating the Quest for Significance™

Partnership with Fluxion Bio

The biggest unmet need is monitoring

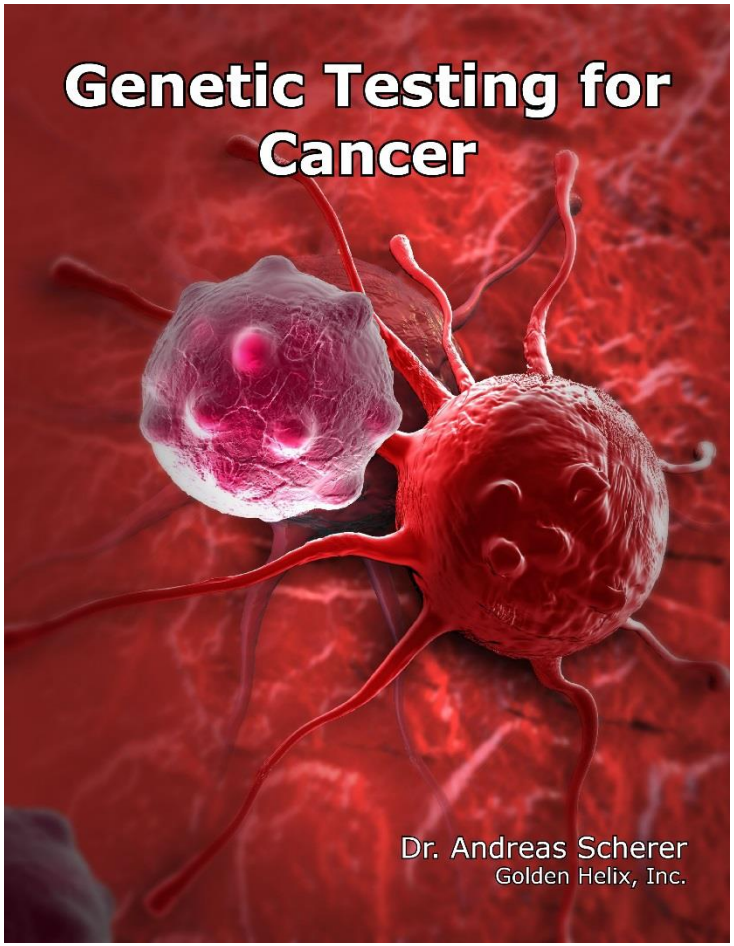


FLUXION

Introductory period for VarSeq ends March 31 2015

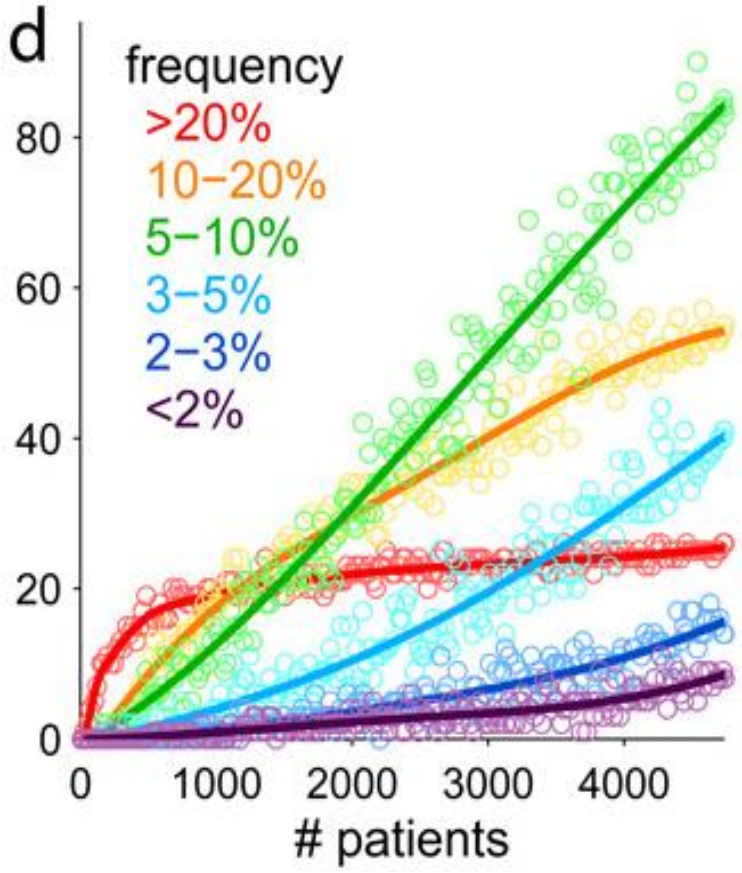
- **Launch in October 2014 covered by GenomeWeb. Just released VarSeq 1.1.1: Gene ranking via PhoRank, Custom Variant Data Base, Log File (Compliance), Note Editor (Reporting)**
- **Presentations at ASHG in October 2014 and TriCon 2015**
- **Customer Announcements**
  - Northshore Next Gen Sequencing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

Resources



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

Outlook



Lawrence et al, Nature 2014

Questions?

Use the Questions pane in your GoToWebinar window