Targeted Pharmacology

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Learning Objectives

1. Discuss the evolution of precision medicine and the associated clinical trial designs
2. Compare and contrast selected diagnostic testing methods and their role in identifying targetable genetic alterations
3. Differentiate between publicly available databases for analysis of genomic information

Evolution of Precision Medicine

- On October 1, 1990, the Human Genome project (HGP) launched
  - International effort to sequence and map all the human genes (~20,000-25,000)
  - Amazing feat was finished in 2003
- In 2006 National Cancer Institute and the National Human Genome Research Institute initiated the landmark Cancer Genome Atlas Program (TCGA)
  - Objective was to molecularly characterize thousands of primary cancers with matched normal samples of 33 cancer types
  - Completed in 2017

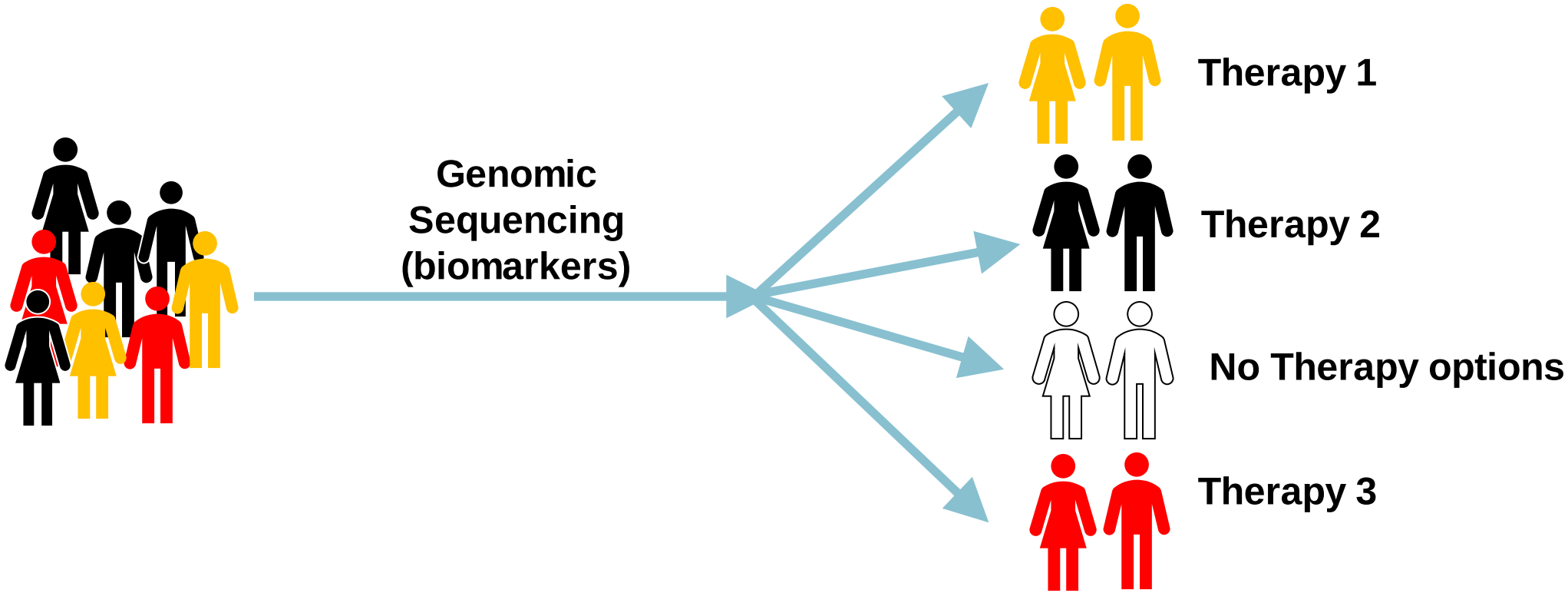
Cancer Science. 2019;110:6–15. | J Exp Clin Cancer Res. 2018;37(1):47.

National Institute of Health (NIH) Initiatives

- In 2015, President Barack Obama & NIH proposed the "Precision Medicine Initiative"
  - Initiative primarily focusing precision & targeted therapies for oncology disease states
- In 2018, the NIH launched their "All of Us" Initiative
  - Goal is to gather both demographic & biological data from one million people in the United States for precision care in oncology

Cancer Science. 2019;110:6–15. | J Exp Clin Cancer Res. 2018;37(1):47.

NIH Precision Medicine Initiative



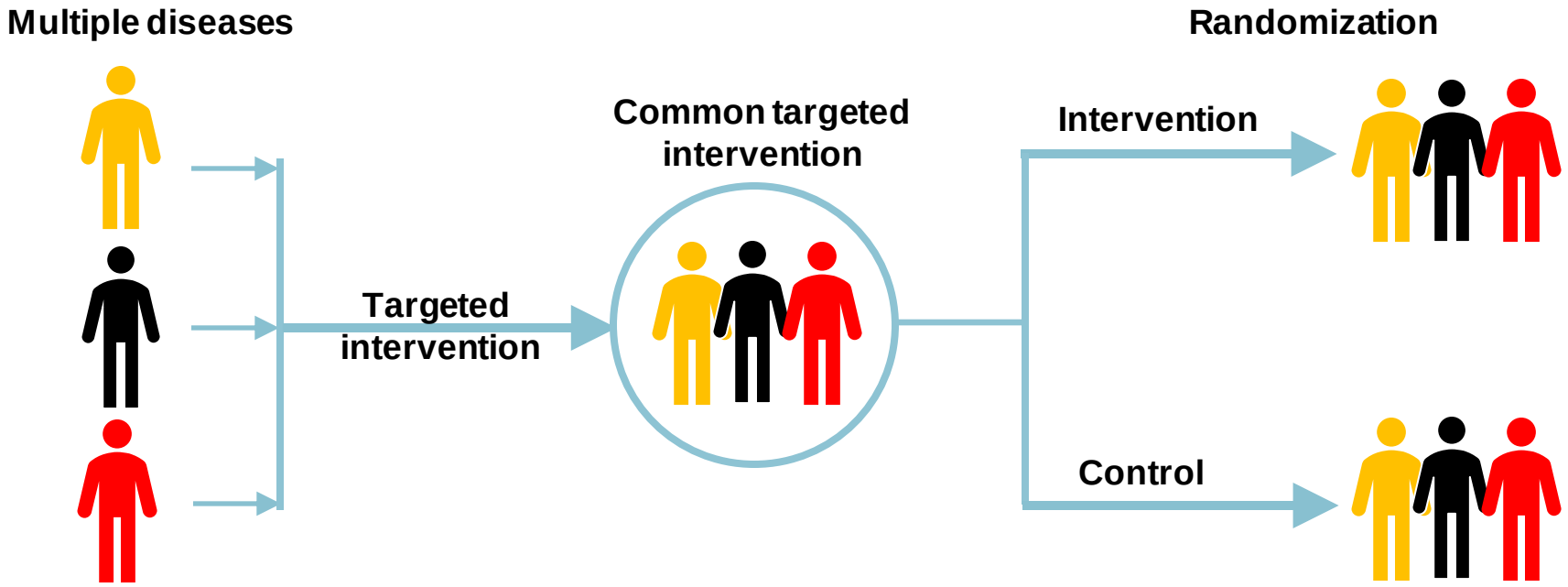
Cancer Science. 2019;110:6–15. | J Exp Clin Cancer Res. 2018;37(1):47.

Clinical Trial Design in Precision Oncology

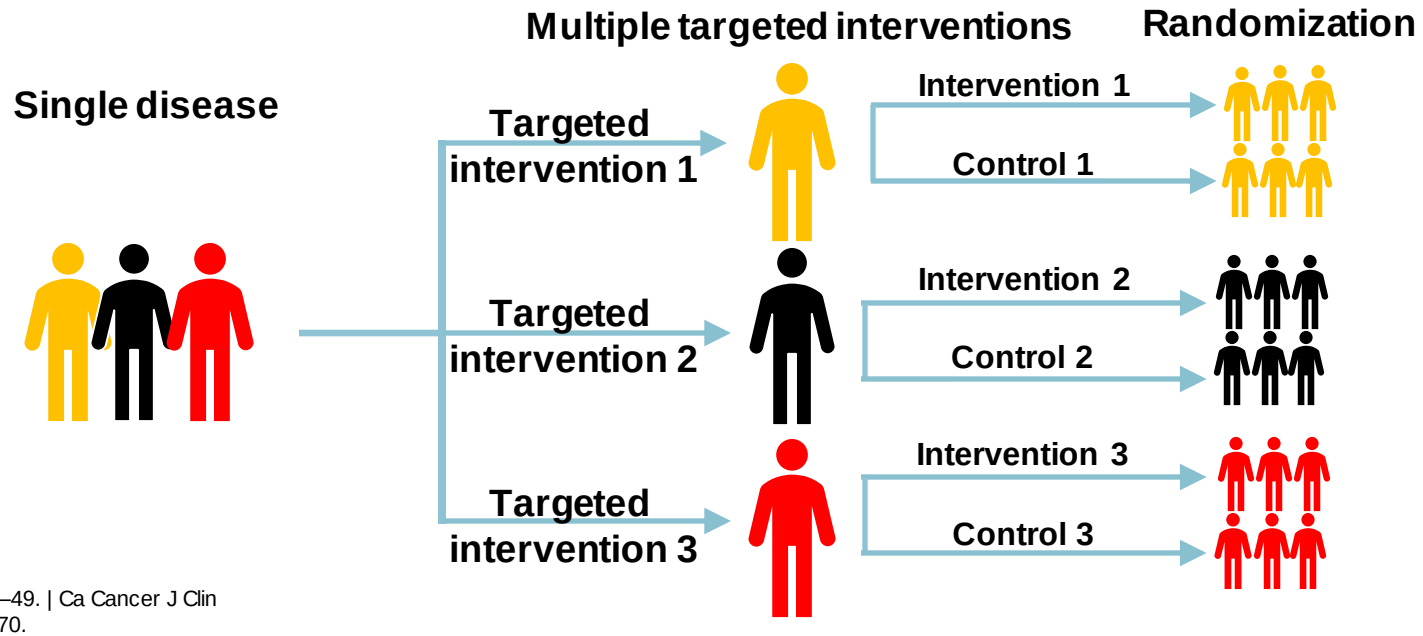
- A master protocol is a single, overarching design developed to evaluate multiple hypotheses
  - Improve efficiency through standardized trial procedures
  - Basket trial: trial designs looking at **one** targeted therapy in **multiple** diseases that share common molecular abnormality
  - Umbrella trial: trial designs looking at **multiple targeted therapies** in **one** disease that is stratified into multiple subgroups (tumor profiling)
  - In 2009 there were only 2 published master trials, and this increased to 67 in 2019 (mostly conducted by National Cancer Institutes

Surg Oncol Clin N Am. 2020 January ; 29(1): 35–49. | Ca Cancer J CLlin2020;70:125–137 | N Engl J Med. 2017;377:62-70.| Trials. 2019;20:572.

Basket Trial



Umbrella Trial



Surg Oncol Clin N Am. 2020 January ; 29(1): 35–49. | Ca Cancer J Clin 2020;70:125–137 | N Engl J Med. 2017;377:62-70.

Basket and Umbrella Trials

- Basket trial examples
  - Pembrolizumab for mismatch repair (MMR)-deficient tumors
    - Two phase II trials showed efficacy across all MMR-deficient solid tumors
    - In 2017, FDA approved it as first tissue agnostic drug (2020 = TMB-H)
  - Larotrectinib/entrectinib for *TRK*-fusion (+) tumors
    - In 2018, FDA approved Larotrectinib as second tissue agnostic drug
    - In 2019, FDA approved entrectinib
  - Vemurafenib in *BRAF*^{v600} mutated malignancies
- Umbrella trial example
  - BATTLE study in non-small cell lung cancer

Surg Oncol Clin N Am. 2020 January ; 29(1): 35–49. | Ca Cancer J CLlin2020;70:125–137 | N Engl J Med. 2017;377:62-70.

Audience Response Question #1

A study evaluating a targeted therapy for a mutation in the *EGFR* gene across multiple disease states most closely represents which of the following?

1. Umbrella trial
2. Randomized controlled trial
3. Basket trial
4. Non-inferiority trial

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Overview of Diagnostic Testing Methods

- Immunohistochemistry (IHC)
- Flow cytometry
- Fluorescent in situ hybridization (FISH)
- Polymerase chain reaction (PCR) and next generation sequencing (NGS)
  - Mutations (missense, nonsense, silent)
    - Somatic versus germline
  - Insertions & deletions

Immunohistochemistry (IHC) & Flow Cytometry

- IHC (biopsy-based)
  - Employs monoclonal/polyclonal antibodies to elucidate **tissue** distribution from biopsy of various membrane and cytoplasmic antigens
  - Microscopy-based (light or fluorescent microscope)
  - Examples: BRAF V600E mutation, HER2 status, PD-L1 expression
- Flow cytometry (fluid/aspirate)
  - Detects and measures size, shape, complexity, and immunophenotype of a cell population
  - Rapid multi-parametric analysis of a cell **fluid** mixture employing laser as a light source to count and sort the cell population
  - Fluorescently conjugated antibodies
  - Examples: CD20, CD22, CD79b

Arch Pathol Lab Med. 2004;128(9):1004-1022.

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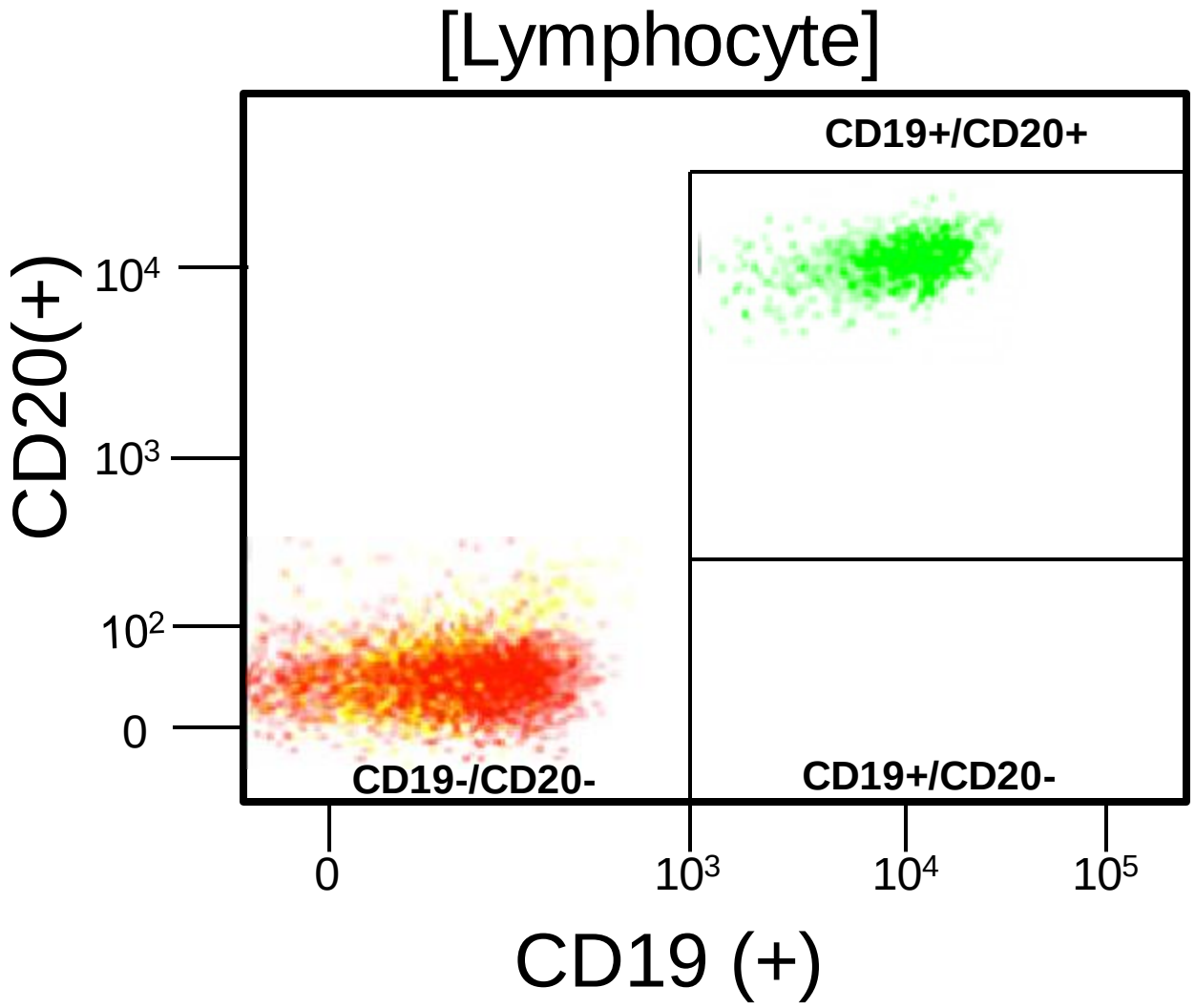
Arch Pathol Lab Med. 2004;128(9):1004-1022.

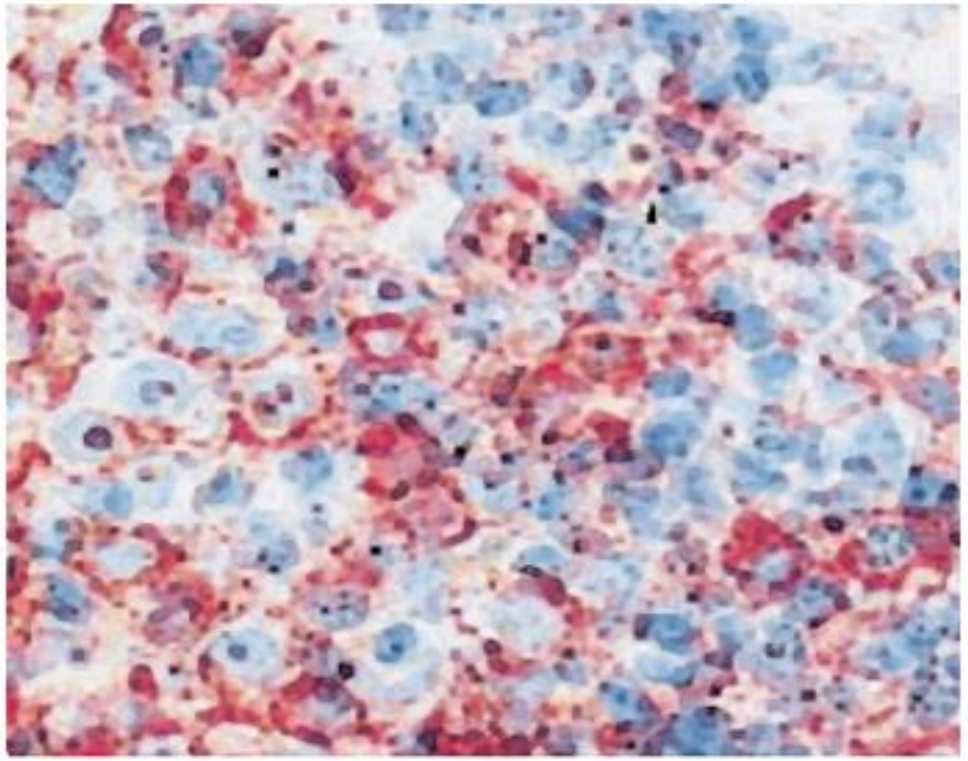
Immunohistochemistry (IHC) & Flow Cytometry

As HER2 overexpression/amplification increases, so does benefit from anti-HER-directed therapy (trastuzumab deruxtecan for HER2 IHC1/2+ & FISH negative)

Semin Cancer Biol. 2021;72:123-135.

Immunohistochemistry (IHC) & Flow Cytometry

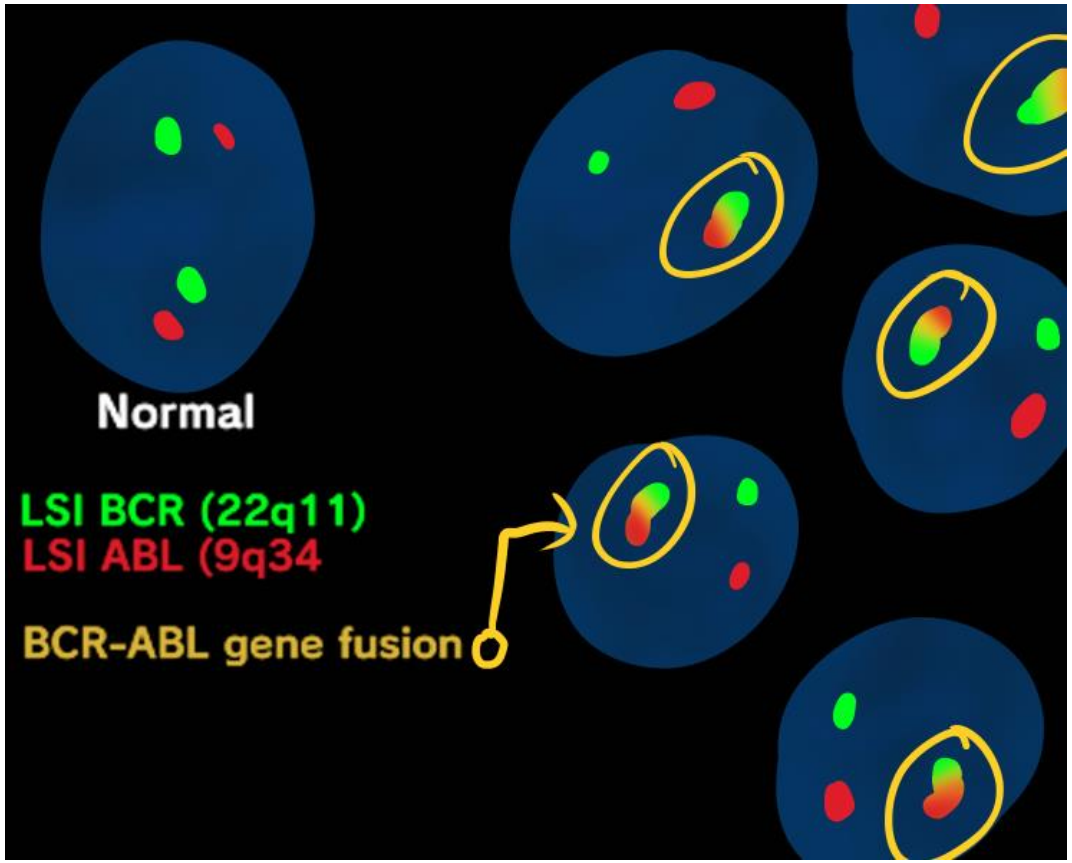




Blood. 2003;102(10):3514-3520.

Fluorescent in situ hybridization (FISH)

- Technique used to identify large chromosomal abnormalities
  - Translocations
  - Duplications
  - Deletions
- Fluorescently labelled DNA probes hybridize with complementary target region and are visualized via fluorescence microscopy



Curr Opin Genet Dev. 1995;5(3):304.

Polymerase chain reaction (PCR) and Next Generation Sequencing (NGS)

- Nucleic acid biochemistry
  - Building blocks for deoxyribose nucleic acid (DNA) and ribose nucleic acids (RNA)
  - Consist of four different bases
    - Sugar
    - Phosphate
    - Nitrogen-containing base
  - Written as 5' → 3' (phosphate on 5' and hydroxyl on 3')
  - Require purine-pyrimidine base pairing (A:T & G:C)

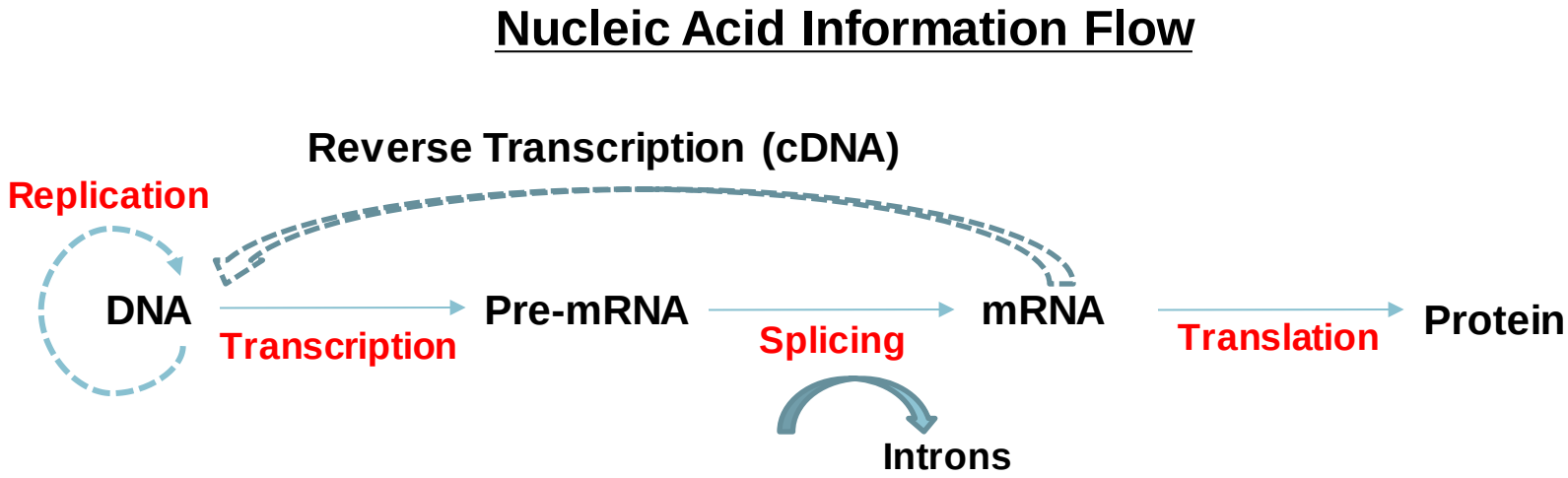
DNA	RNA	
Adenine	Adenine	
Guanine	Guanine	Purines
Cytosine	Cytosine	
Thymine	Uracil	Pyrimidines

5' – TCGATATATC – 3'
3' – AGCTATATAG – 5'

Complementary Sequences

Greenspan, Daniel. (2003). From Genes to Genomes: Concepts and Applications of DNA Technology.
Tan, D., & Lynch, H. T. (2013). Principles of molecular diagnostics and personalized cancer medicine.

Polymerase chain reaction (PCR) and Next Generation Sequencing (NGS)



- Central dogma of molecular biology → flow of genetic information
- Complex processes requiring a myriad of proteins (e.g. scaffolding proteins, enzymes, etc.)
- Final product is protein

mRNA: messenger RNA
cDNA: complementary DNA

Greenspan, Daniel. (2003). From Genes to Genomes: Concepts and Applications of DNA Technology.
Tan, D., & Lynch, H. T. (2013). Principles of molecular diagnostics and personalized cancer medicine.

Polymerase chain reaction (PCR) and Next Generation Sequencing (NGS)

- Molecular technique that amplifies a targeted nucleic acid region for sequencing or quantification
  - Sequencing
    - Sanger sequencing
    - Next generation sequencing (NGS)
  - Quantification
    - Real time (quantitative) PCR
- DNA can be directly amplified
- RNA is converted back to cDNA using reverse transcriptase
  - Exons only (common technique for fusion protein transcript analysis)

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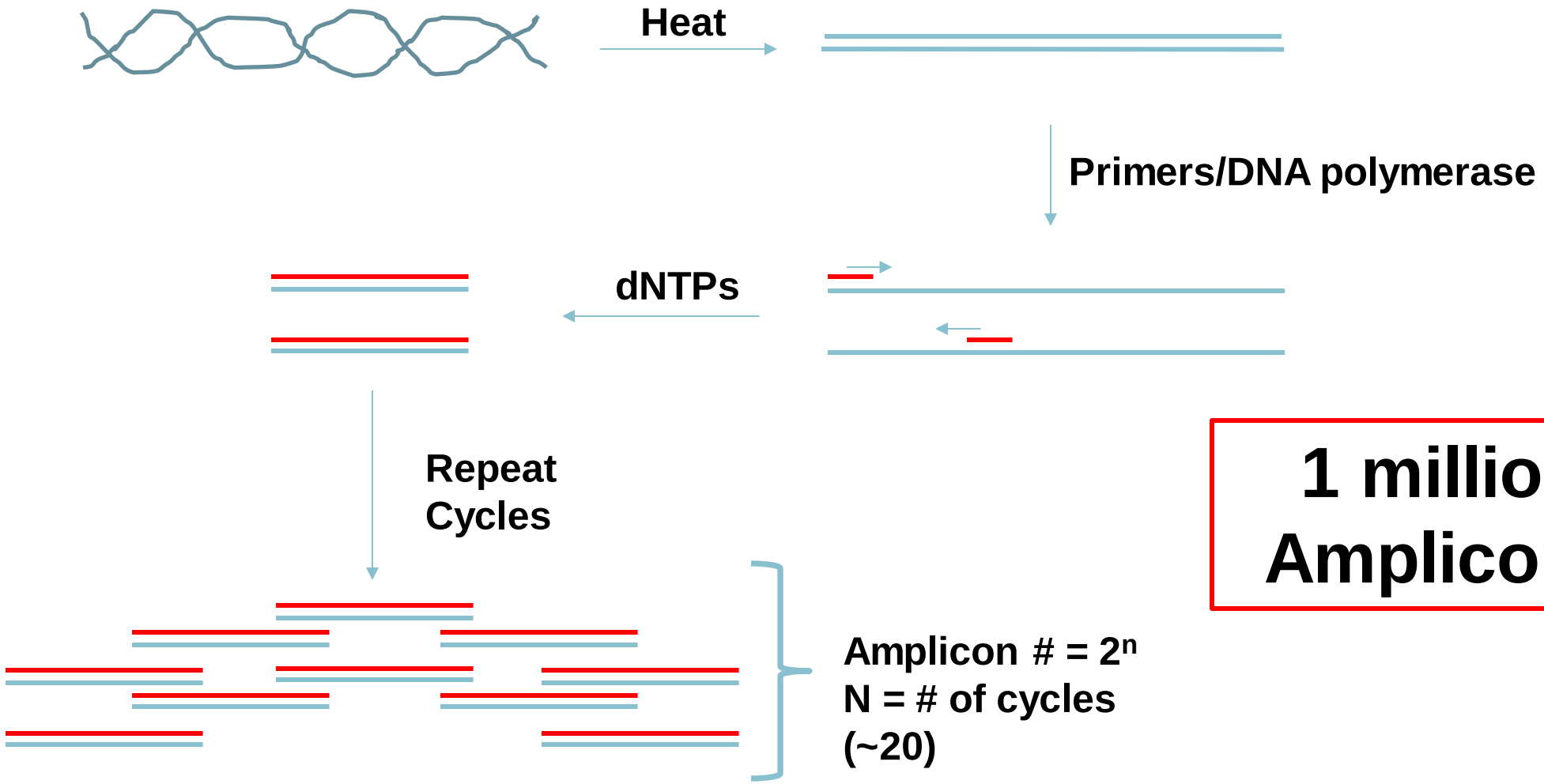
Polymerase Chain Reaction (PCR)



Greenspan, Daniel. (2003). From Genes to Genomes: Concepts and Applications of DNA Technology.

Polymerase chain reaction (PCR) and Next Generation Sequencing (NGS)

- Sequencing DNA is a way to determine nucleic acid base order
  - Sanger Sequencing
    - Target sequencing of one gene (e.g. FLT3-TKD)
    - Sequence length of 500-800 base pairs
    - Most institutions can run
  - NGS (RNA or DNA based)
    - High-throughput sequencing/deep sequencing
    - Sequence hundreds of genes simultaneously
    - Sequence length ~200-500 base pairs
    - Usually send out (takes 2-3 weeks)

Gives a targeted answer to a specific question

Gives a complete picture of disease clonality

Greenspan, Daniel. (2003). From Genes to Genomes: Concepts and Applications of DNA Technology.
Tan, D., & Lynch, H. T. (2013). Principles of molecular diagnostics and personalized cancer medicine.

Quick Dive into NGS

- Nucleic acid isolation → Library preparation of fragments →clonal amplification and sequencing → Computational/data analysis
  - Computational output using bioinformatics involves processing, analysis, and interpretation of variants

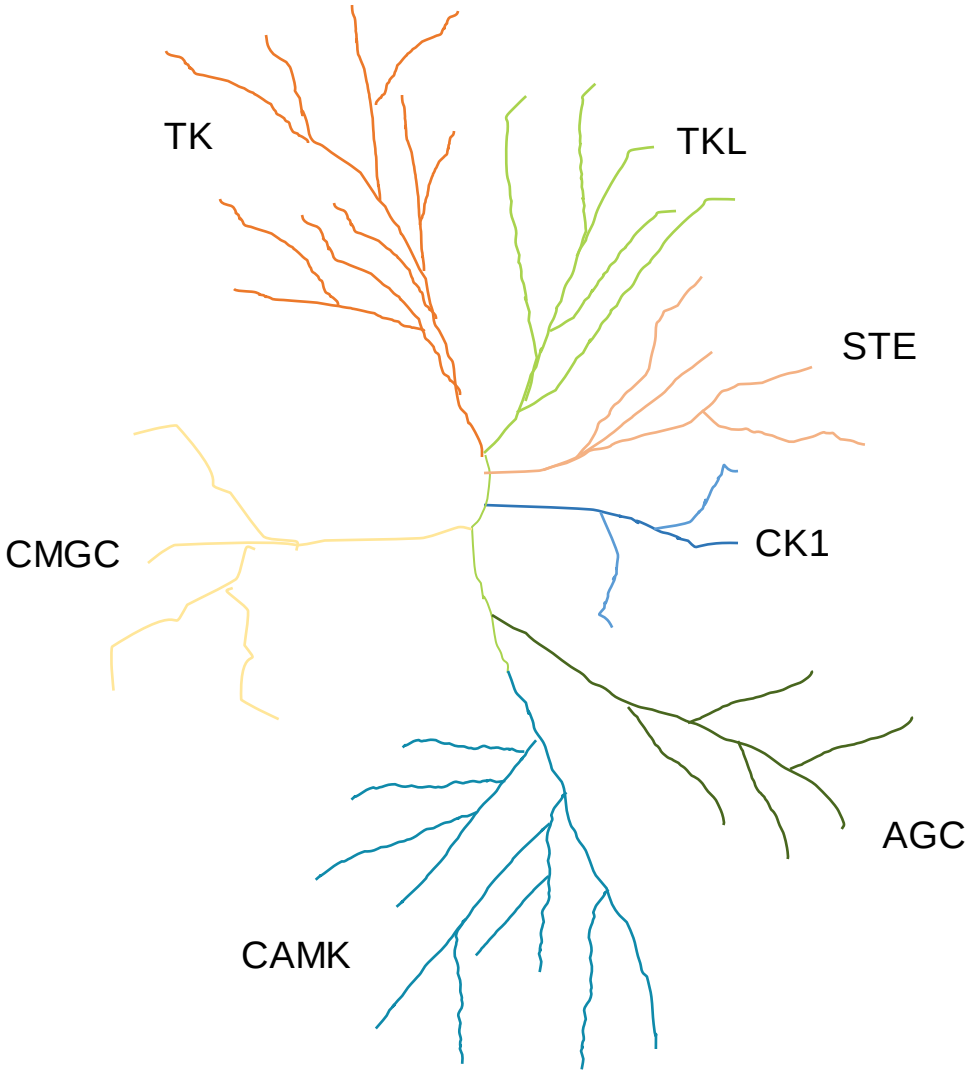
Output of Variant Caller

Gene	Mutation	Protein	Class	Variant Allele Frequency
ALK	c.3824G>T	p.R1275L	MISSENSE	35%
BRAF	c.1799T>A	p.V600E	MISSENSE	52%
NPM1	c.860_863dupTCTG	p.W288Cfs*12	FRAMESHIFT	40%

- Supporting literature and interpretation may be provided in report
  - Tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants of unknown clinical significance

Greenspan, Daniel. (2003). From Genes to Genomes: Concepts and Applications of DNA Technology.
Tan, D., & Lynch, H. T. (2013). Principles of molecular diagnostics and personalized cancer medicine.

In Vitro Kinase Selectivity of Bruton Tyrosine Kinase Inhibitors



Kinase	Ibrutinib	Acalabrutinib	Zanubrutinib
BTK	1.5	5.1	0.5
TEC	10	126	44
ITK	4.9	> 1000	50
BMX	0.8	46	1.4
EGFR	5.3	> 1000	21
ERBB4	3.4	16	6.9
JAK3	32	> 1000	1377
BLK	0.1	> 1000	2.5

Kaptein. ASH 2018. Abstr 1871.

Tyrosine Kinase Inhibitor Effectiveness in Mutated BCR-ABL1 in Vitro

	Imatinib	Nilotinib	Dasatinib	Bosutinib	Ponatinib
G250E	6.37	4.74	1.51	3.93	5.15
Y253H	15.95	34.89	1.84	0.91	5.28
E255V	10.98	27.6	3.76	2.65	11.28
V299L	1.83	1.81	10.33	13.65	1.22
T315I	27.7	266.76	806.32	17.06	5.59
F317L	3.49	2.93	6.59	2.32	1.53
F359V	4.0	7.58	1.45	1.04	4.7
F486S	5.35	2.68	2.2	2.31	2.05

	< 2 = Vulnerable
	2-6 = Slightly resistant
	6-12 Moderately resistant
	12+ Highly resistant

Sci Rep. 2022;12(1):5164.

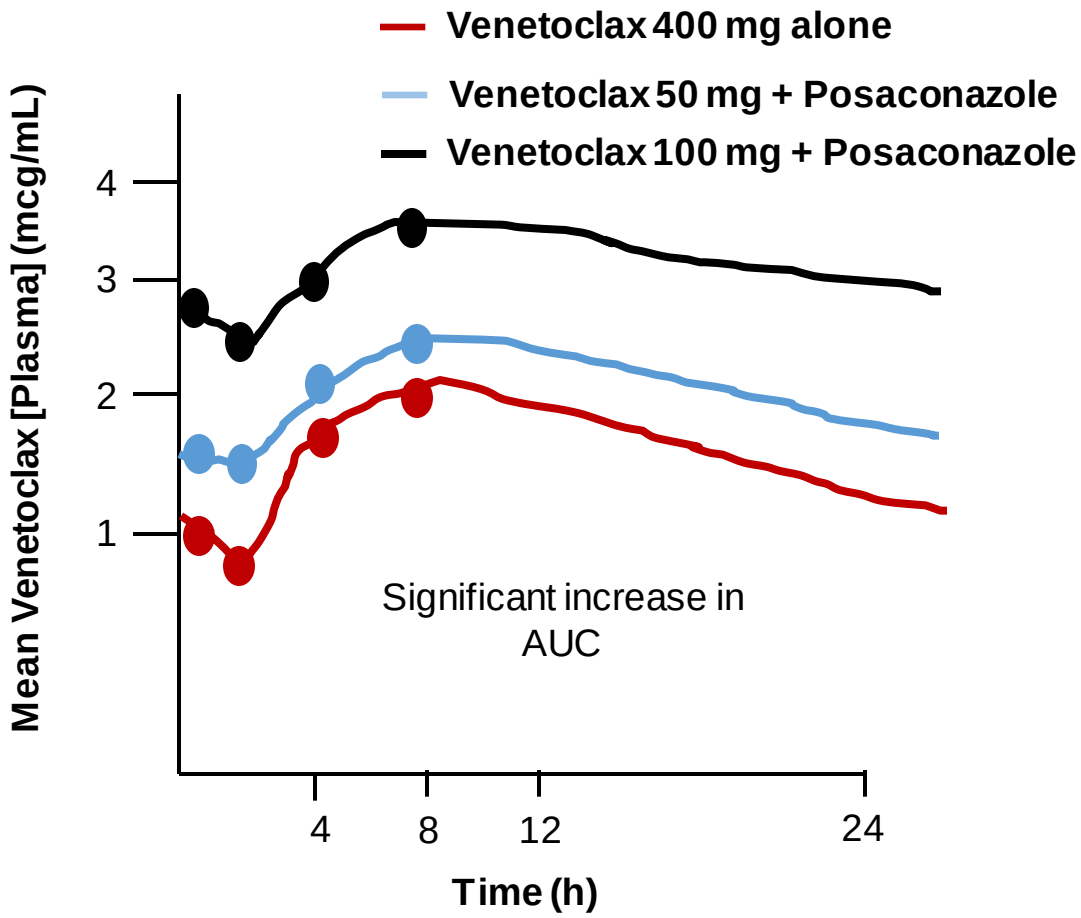
Compound vs Polyclonal mutations

- Compound mutation
  - Two or more mutations in the same molecule
  - *BCR-ABL1:* multiple missense mutations (e.g E255V & T315I) within same sample & same protein
  - Impact on TKI sensitivity → changes structure activity relationship (SAR) (IC50 NOT maintained)
- Polyclonal mutation
  - Mutations present in different molecules
  - *BCR-ABL1:* multiple missense mutations (e.g. E255V & T315I) within same sample but DIFFERENT fusion proteins
  - Impact on TKI sensitivity → NO change to SAR (IC50 maintained)
- How to determine compound vs polyclonal mutation in CML
  - Two possible ways by NGS
  - May not be feasible

Blood. 2013;121(3):489-498.

Drug Interactions with Targeted Therapy

- Marker for response
  - PCR
  - Tumor response on scans
- Package insert or interaction database
  - Detailed instructions
  - Alternatives
  - Fold change in AUC
    - Posaconazole + venetoclax = 8.8-fold ↑ AUC



Clin Ther. 2017;39(2):359-367.

Selected Targeted Therapies

- Non-small cell lung cancer
  - EGFR, ALK, ROS1, RET, MET, and PD-L1 inhibitors
- Ovarian Cancer
  - PARP inhibitors
- Breast Cancer
  - Anti-HER2 monoclonal antibodies
- Melanoma
  - BRAFV600E/MEK inhibitors
- Acute myeloid leukemia
  - FLT3-ITD, FLT3-TKD, IDH1, and IDH2 inhibitors
- B-cell Non-Hodgkin lymphoma (B-NHL)
  - Anti-CD20 monoclonal antibodies, EZH2 inhibitor
- B-acute lymphoblastic leukemia (B-ALL)
  - CD3xCD19 (BiTE therapy), anti-CD22/CD20 monoclonal antibodies, BCR-ABL inhibitors
- Chronic myeloid leukemia
  - BCR-ABL and STAMP inhibitors

Targeted Therapy Success Stories

- Rituximab – Anti-CD20 antibody (2002)
  - R-CHOP vs CHOP
    - Complete response = 76% vs 63%
- Vemurafenib – BRAF V600E inhibitor (2011)
  - Vemurafenib vs Dacarbazine
    - 6-month overall survival = 84% vs 64%
    - 63% reduction in death at interim analysis
- Capmatinib – MET Exon 14 Skipping inhibitor (2020)
  - GEOMETRY mono-1 trial
    - Overall response rate = 68% in treatment naïve cohort

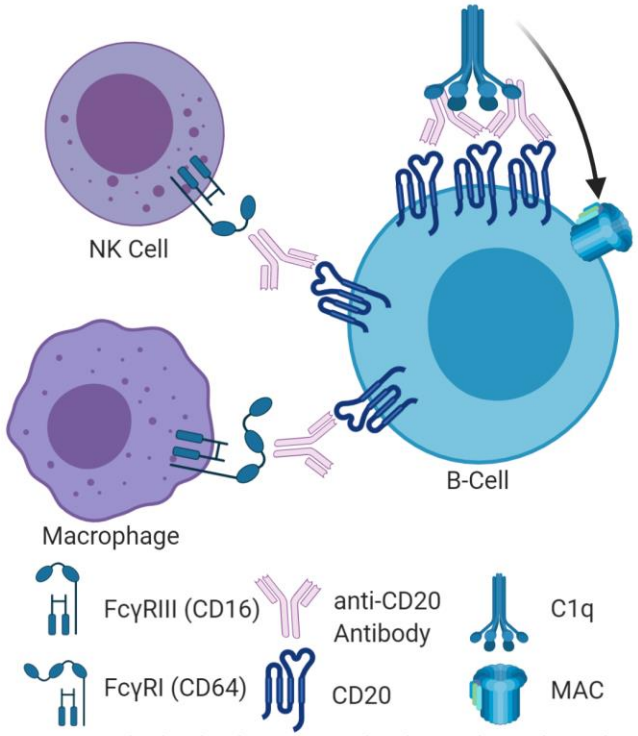


Figure 2: Anti-CD20 antibodies bind to CD20 molecules on the surface of B-cells leading to cell death through antibody-dependent cell-mediated cytotoxicity (ADCC) via FCγRs on macrophages and NK cells or through complement-dependent cytotoxicity (CDC) via activation of the complement cascade and formation of membrane attack complexes (MAC)

Created with BioRender.com.

N Engl J Med 2002; 346:235-242. | N Engl J Med, 2011, 364(26):2507-16 | N Engl J Med 2020; 383:944-957

Master Trials: The Good & The Bad

Vemurafenib in nonmelanoma BRAF V600 mutations

- Phase II "basket" trial encompassing 7 cohorts

Variable	NSCLC (N=20)	Colorectal Cancer Vem (N=10)	Colorectal Cancer Vem+Cet (N=27)	Cholangiocarcinoma (N=8)	ECD/LCH (N=18)	Anaplastic Thyroid cancer (N=7)
CR (%)	0	0	0	0	1 (7)	1 (14)
PR (%)	8 (42)	0	1 (4)	1 (12)	5 (36)	1 (14)
SD (%)	8 (42)	5 (50)	18 (69)	4 (50)	8 (57)	0
ORR (%)	8 (42)	0	1 (4)	1 (12)	6 (43)	2 (29)

N Engl J Med. 2015;373(8):726-736.

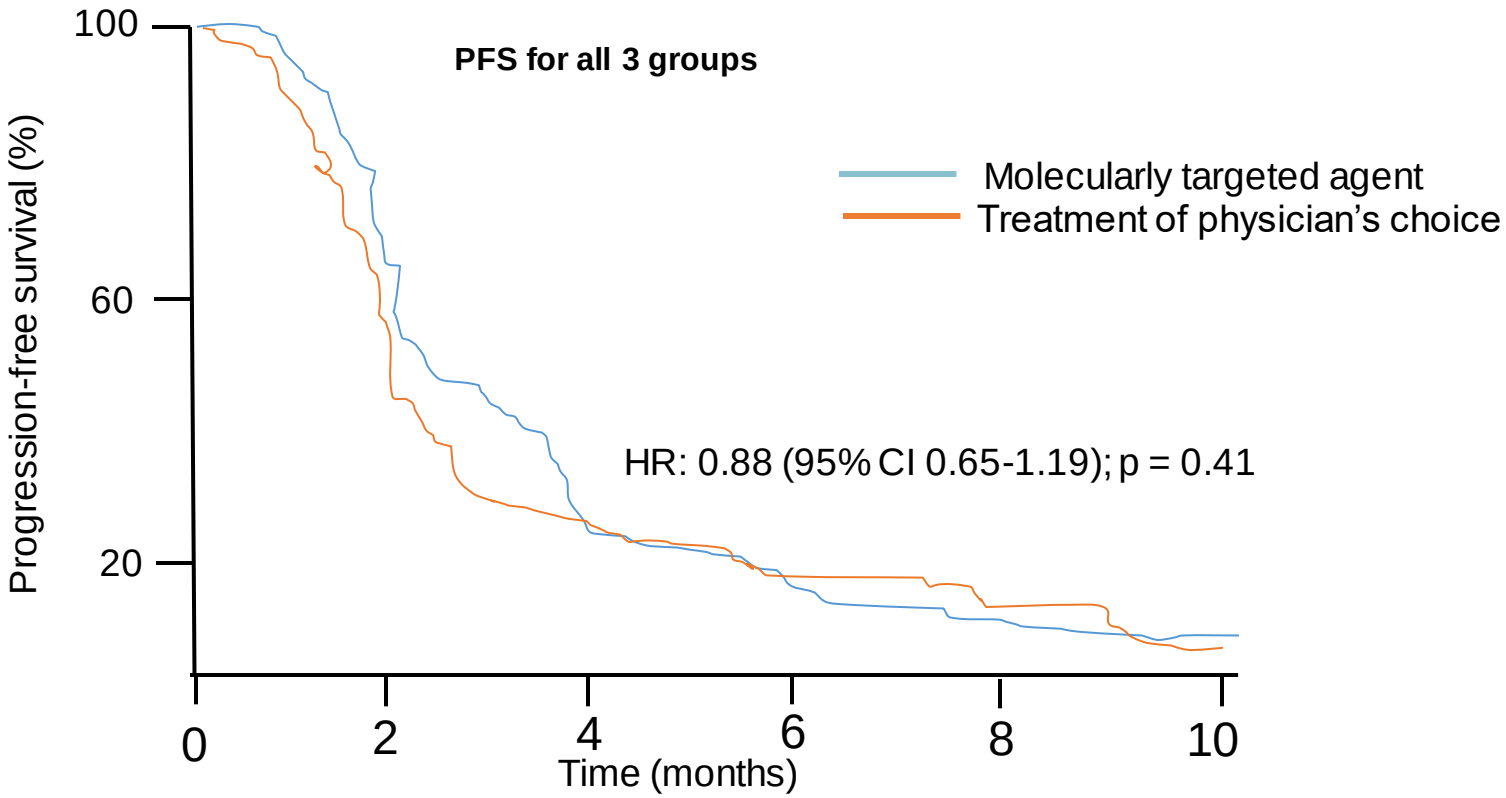
Master Trials: The Good & The Bad

- SHIVA Trial: targeted therapy vs conventional therapy
  - Open-label, randomized, controlled phase 2 trial (France)
  - Adult patients with metastatic solid tumor refractory to standard of care
  - Molecular profile completed of the tumors
    - Must have mutation in one of the three pathways: hormone receptor, PI3K/AKT/mTOR, or RAF/MEK
    - These mutations were matched to one of ten regimens (erlotinib, lapatinib plus trastuzumab, sorafenib, imatinib, dasatinib, vemurafenib, everolimus, abiraterone, letrozole, tamoxifen)
  - Randomized to matched molecularly targeted therapy (outside of indication) or physicians' choice

Lancet Oncol. 2015;16(13):1324-1334.

Master Trials: The Good & The Bad

SHIVA Trial: targeted therapy vs conventional therapy



- Hormone receptor pathway
  - PFS HR 1.12; p = 0.64
- PI3K/AKT/mTOR pathway
  - PFS HR 0.79; p = 0.30
- RAF/MEK pathway
  - PFS HR 0.58; p = 0.20

Lancet Oncol. 2015;16(13):1324-1334.

Audience Response Question #2

Your institute is planning on enrolling patients for a clinical trial for targeted therapy for a newly discovered protein, T3FB1, with a V445S mutation. What test would be the most appropriate to screen for the presence of this mutation?

1. Quantitative RT-PCR
2. Flow cytometry
3. NGS
4. PCR w/ Sanger Sequencing

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Public databases for Interpretation of Genomic Information

- Databases for clinical actionability
  - OncoKB (https://www.oncokb.org/actionableGenes#sections=Tx)
  - CIViC (https://civicdb.org/welcome)
  - cBioPortal (https://www.cbioportal.org/)
- Genetic databases
  - COSMIC (https://cancer.sanger.ac.uk/cosmic)
  - gnomAD (including ExAC) (https://gnomad.broadinstitute.org/)
  - ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)

Public Databases for Interpretation of Genomic Information

Databases for clinical actionability

- OncoKB
  - Simple, comprehensive, and user-friendly database out of Memorial Sloan Kettering
- CIViC
  - Complicated, extensive, and technically difficult database
  - Complex molecular profiles (multiple genes included with multiple variants to get evidence)
  - Gene mutation 1 AND gene mutation 2 AND gene mutation (n)… vs simple molecular profile which is 1 variant or one gene mutation (BRAFV600E)
  - Includes fusion proteins (e.g. P2RY8::CRLF2 case reports)
- cBioPortal
  - Supports a range of –omic data (e.g. mutations, fusions, mRNA expression, RNA-seq, DNA methylation, etc.)
  - Wide range of clinical data (e.g. treatments, survival/outcomes, biomarkers)
  - Background biological data (e.g. 2D and 3D protein structures with associated mutations)
  - Includes a variety of other knowledgebase resources such as OncoKB and CIViC (curated effect and therapy implications), variant recurrence (COSMIC) amongst others

Public databases for Interpretation of Genomic Information

Genetic databases

- COSMIC
  - Catalogue of somatic mutations in cancer including rare gene mutations
  - Includes clinical case reports along with pathology articles/data sets
  - Good resource to see if specific mutations have previously been reported
- ClinVar
  - NIH resource for assessing pathogenicity of reported variants & assessment of variants of “unknown significance”
  - Three letter amino acid designation needed (e.g. TP53 R249S = TP53 Arg249Ser)
    - Germline testing required? Can utilize National Comprehensive Cancer Network Guidelines

Audience Response Question #3

What is the best public database for finding literature on complex molecular profiles (i.e. multiple mutations within a tumor sample)?

1. OncoKB
2. ClinVar
3. BRCA Exchange
4. CIViC

Patient Case

- 55 yo female with newly diagnosed acute leukemia
  - Presented from OSH with weakness, melena, fever
  - Found to have leukocytosis and peripheral smear with circulating blasts
  - Smear raises concern for APL vs AML – ATRA started empirically
  - Bone marrow biopsy to be completed for full work up

- Several studies will be sent off for confirmation of diagnosis

Patient Case

Which of the following assays can be run to rapidly rule in or rule out APL (translocation of chromosomes 15 and 17)?

1. FISH
2. Flow cytometry
3. Chromatin immunoprecipitation (CHIP)
4. DNA microarray analysis

Patient Case

- 55 yo female with newly diagnosed acute leukemia
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  - Found to have leukocytosis and peripheral smear with circulating blasts
  - ~~Smear raises concern for APL~~ vs AML
  - Bone marrow biopsy to be completed for full work up

- FISH for t(15;17) negative, so tretinoin stopped
- Peripheral blood flow cytometry confirms AML
- Results from bone marrow biopsy pending

Patient Case

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  - Presented from OSH with weakness, melena, fever
  - Found to have leukocytosis and peripheral smear with circulating blasts
  - ~~Smear raises concern for APL~~ vs AML
  - Bone marrow biopsy to be completed for full work up

- Based on peripheral blood flow cytometry showing 50% circulating myeloblasts, standard chemotherapy induction started with 7+3
- Remainder of FISH negative for core binding factor translocations (will not add gemtuzumab ozogamicin to therapy)

Patient Case

- 55 yo female with newly diagnosed acute leukemia
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- Based on peripheral blood flow cytometry showing 50% circulating myeloblasts, standard chemotherapy induction started with 7+3
- Remainder of FISH negative for core binding factor translocations (will not add gemtuzumab ozogamicin to therapy)

Patient Case

DNA was extracted and amplified from the bone marrow biopsy specimen. Using fluorescent PCR and a primer pair flanking two exons of the FLT3 gene, an internal tandem duplication of the FLT3 gene was detected (FLT3-ITD mutant : wild type = 0.55). Which of the following statements is most accurate?

1. Add FLT3 inhibitor regardless of FDA-approval status
2. If an FDA-approved FLT3 inhibitor exists, add to 7+3 backbone
3. There is no assay to detect a FLT3 mutation, so it is not possible to know FLT3 status
4. I am not sure

Patient Case

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  - Presented from OSH with weakness, melena, fever
  - Found to have leukocytosis and peripheral smear with circulating blasts
  - ~~Smear raises concern for APL~~ vs AML
  - Bone marrow biopsy to be completed for full work up

- Diagnosis of FLT3-ITD(+) AML has been confirmed. Full molecular profile pending.
  - Since only looking for single mutation, Sanger Sequencing PCR is gold standard for FLT3-ITD and FLT3-TKD testing

Patient Case

- 55 yo female FLT3-ITD(+) AML, 46XX
  - Additional mutations found through Sanger Sequencing include the following: FLT3-TKD, NPM1-4BP insertion, IDH2 R140
  - NGS panel came back with several more mutations including the following: SF3B1 (VAF 40.95%; Tier 1), KRAS (VAF 12.04%; Tier 2), SMC1A (VAF 5.32%; Tier 3)

- Complete picture of this specific AML with cytogenetics and molecular profile
  - Targeted therapies for this patient include FLT3 inhibitor and IDH2 inhibitor

Conclusion

- Precision medicine in oncology is based on patient-specific factors such as cytogenetics, gene mutations, and other biomarkers that can help identify specific targeted agents such as small molecule drugs and monoclonal antibodies

- Precision medicine led to significant treatment advances and outcomes in a myriad of various malignancies (e.g., breast cancer, ECD, melanoma, and CML)

- The advances in precision medicine have been accomplished through improvements in diagnostic techniques and especially due to high throughput gene sequencing technologies like NGS

Targeted Pharmacology

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