Εφαρμογή της τεχνολογίας NGS στην κλινική πράξη…

…στη διάγνωση αιματολογικών νοσημάτων

Μαρία Γαροφαλάκη

Βιολόγος, Εργαστήριο Μοριακής Βιολογίας,

Αιματολογική-Λεμφωμάτων Κλινική και ΜΜΜΟ

24° Ετήσιο Σεμινάριο Συνεχιζόμενης Ιατρικής Εκπαίδευσης Νοσοκομείου «Ο Ευαγγελισμός»

Αθήνα, 18 - 22 Φεβρουαρίου 2019

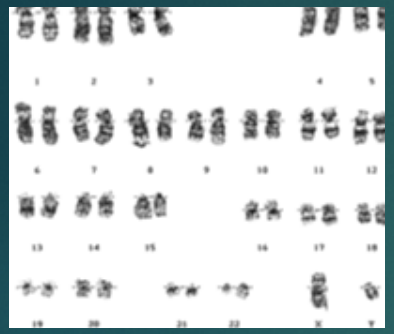
Δεν υπάρχει σύγκρουση συμφερόντων
με τις παρακάτω χορηγούς εταιρείες:

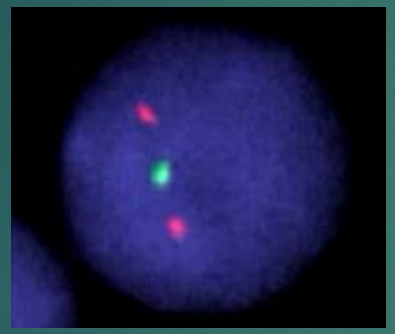
PFIZER, JANSSEN ONCOLOGY, SOFMEDICA,
NOVARTIS, ABBVIE, MSD, WINMEDICA,
GENESIS, ROCHE, TAKEDA, ASTELLAS,
AMGEN, ANGELINI, ΑΝΤΙΣΕΛ, SERVIER,
BRISTOL-MYERS SQUIBB, ABBOTT, GILEAD,
SANDOZ, BIANEΞ, RONTIS, MAVROGENIS,
AENORASIS, SPECIFAR, KARYO

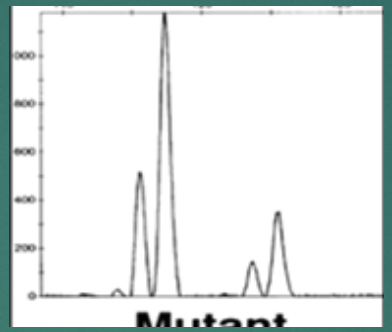
Γιατί γενετική διάγνωση στην αιματολογία;

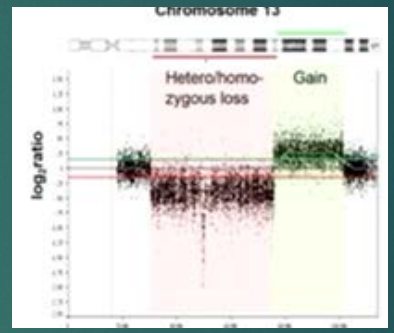
- Διάγνωση
- Πρόγνωση – διαστρωμάτωση κινδύνου
- Θεραπευτικές επιπτώσεις
- Παρακολούθηση της εξέλιξης της Νόσου
- Ανίχνευση ΕΥΝ

Εξέλιξη της γενετικής διαγνωστικής





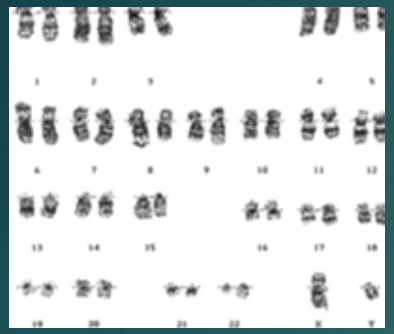


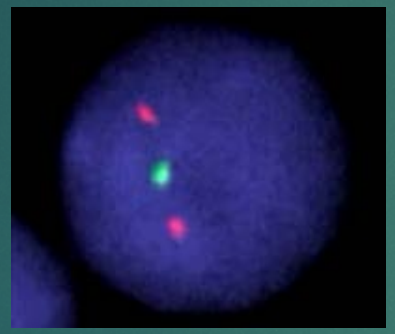


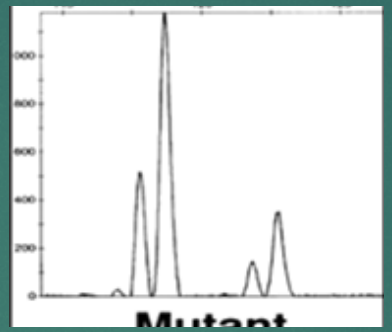
- G-Banding
- FISH
- PCR
- microarrays

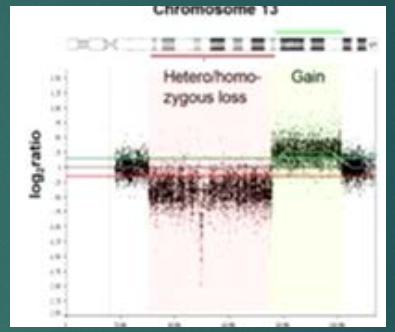
1970 2010

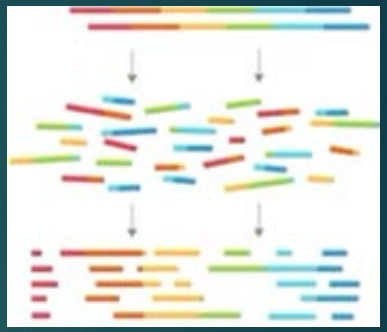
Εξέλιξη της γενετικής διαγνωστικής











- G-Banding
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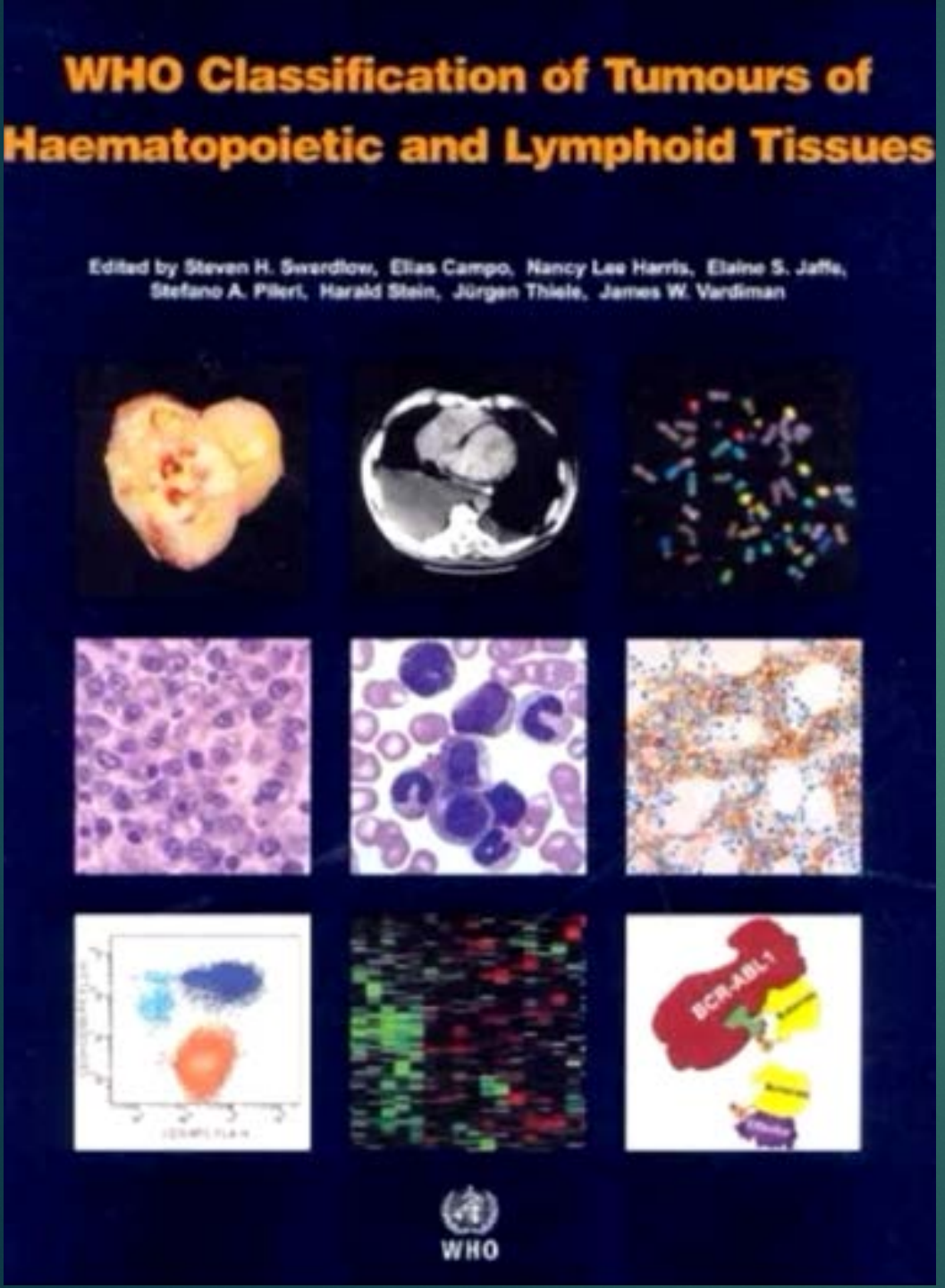
- NGS

1970 2010


WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues
Edited by Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe, Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman
BCR-ABL1
WHO

Κατάταξη WHO (π.χ.ΟΜΛ)

Acute myeloid leukemia (AML) and related neoplasms

- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
  - APL with *PML-RARA*
  - AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
  - AML with t(6;9)(p23;q34.1);*DEK-NUP214*
  - AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
  - AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
  - Provisional entity: AML with *BCR-ABL1*
  - AML with mutated *NPM1*
  - AML with biallelic mutations of *CEBPA*
  - Provisional entity: AML with mutated *RUNX1*
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, NOS
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Pure erythroid leukemia
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis (TAM)
  - Myeloid leukemia associated with Down syndrome

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Myeloid neoplasm classification

Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction

- AML with germ line *CEBPA* mutation
- Myeloid neoplasms with germ line *DDX41* mutation*

Myeloid neoplasms with germ line predisposition and preexisting platelet disorders

- Myeloid neoplasms with germ line *RUNX1* mutation*
- Myeloid neoplasms with germ line *ANKRD26* mutation*
- Myeloid neoplasms with germ line *ETV6* mutation*

Myeloid neoplasms with germ line predisposition and other organ dysfunction

- Myeloid neoplasms with germ line *GATA2* mutation
- Myeloid neoplasms associated with BM failure syndromes
- Myeloid neoplasms associated with telomere biology disorders
- JMML associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
- Myeloid neoplasms associated with Down syndrome*

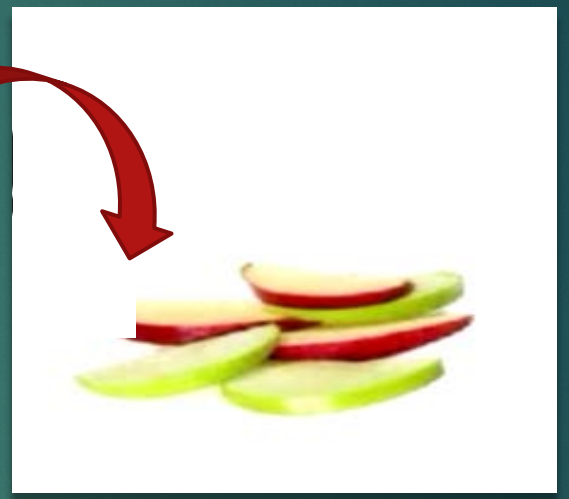
Νέο εργαλείο για διαγνωστική & έρευνα

Whole Genome Seq

3 000 000 000 pb

Whole Exome Seq

21 000 genes



Targeted panels

100 genes

WGS, WES, Gene panels: χρησιμοποιούνται στα εργαστήρια

NGS

Targeted panels	WES	WGS
RNA Seq	Immune Seq	CHIP Seq Methylome Seq

Γιατί Next-Gen ;;;

ιατρική ακριβείας

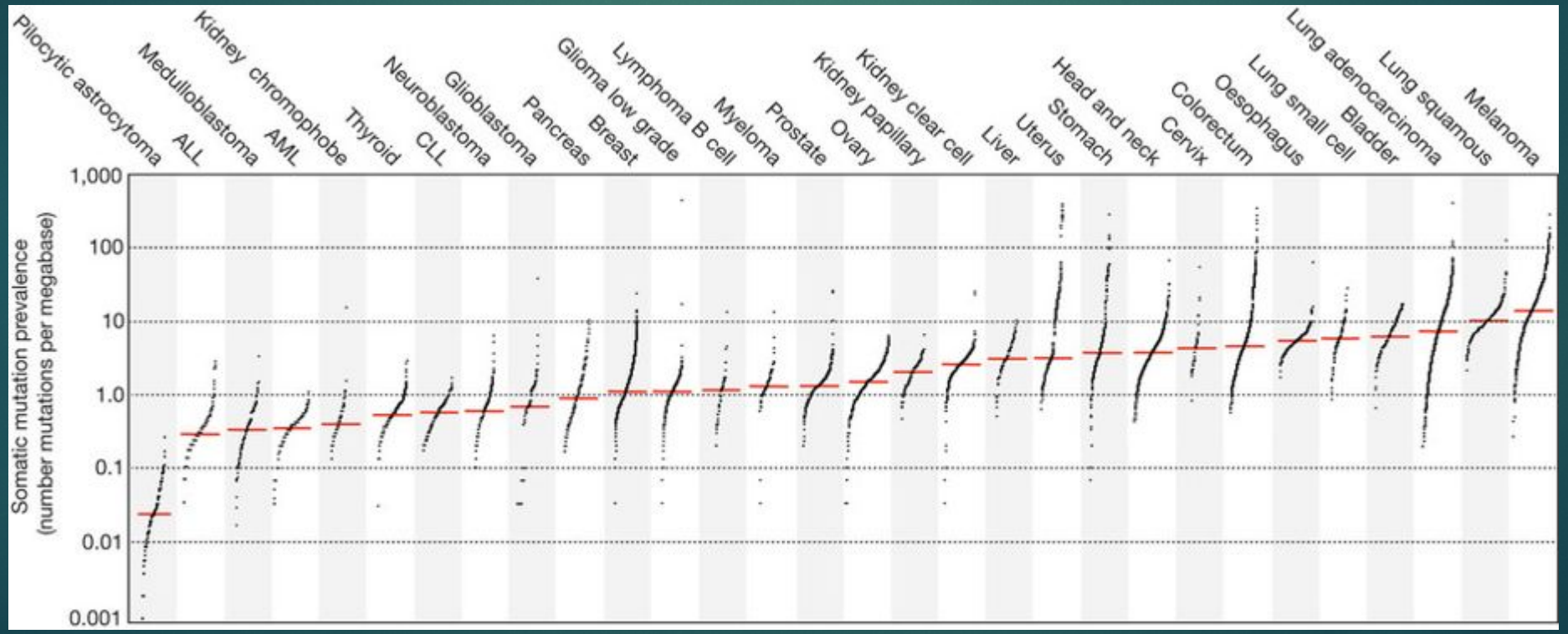
Τι είναι η ιατρική ακριβείας ;

ΓΙΑ ΤΟΝ ΚΛΙΝΙΚΟ ΓΙΑΤΡΟ

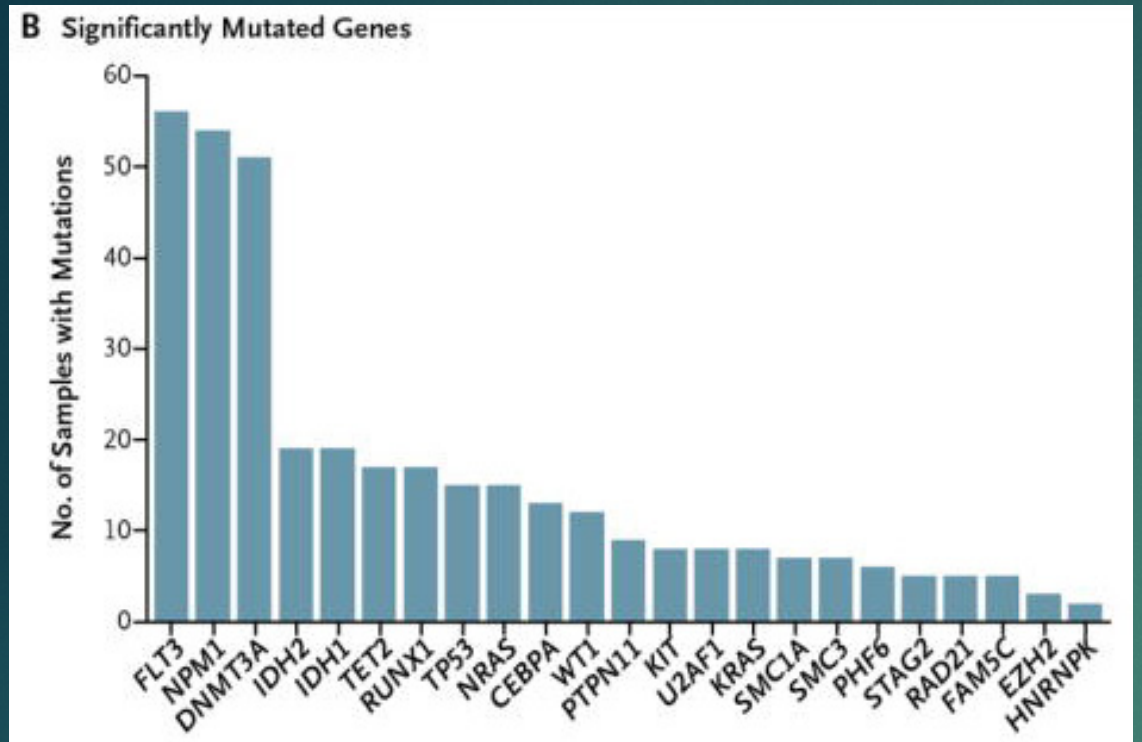
εξατομικευμένη ιατρική περίθαλψη με βάση τα ατομικά χαρακτηριστικά των ασθενών, και την κατάταξή τους σε υποομάδες με βάση παράγοντες όπως η επιθετικότητα της ασθένειας ή η προβλεπόμενη ανταπόκριση στη θεραπεία

Φορτίο μεταλλάξεων/καρκίνο

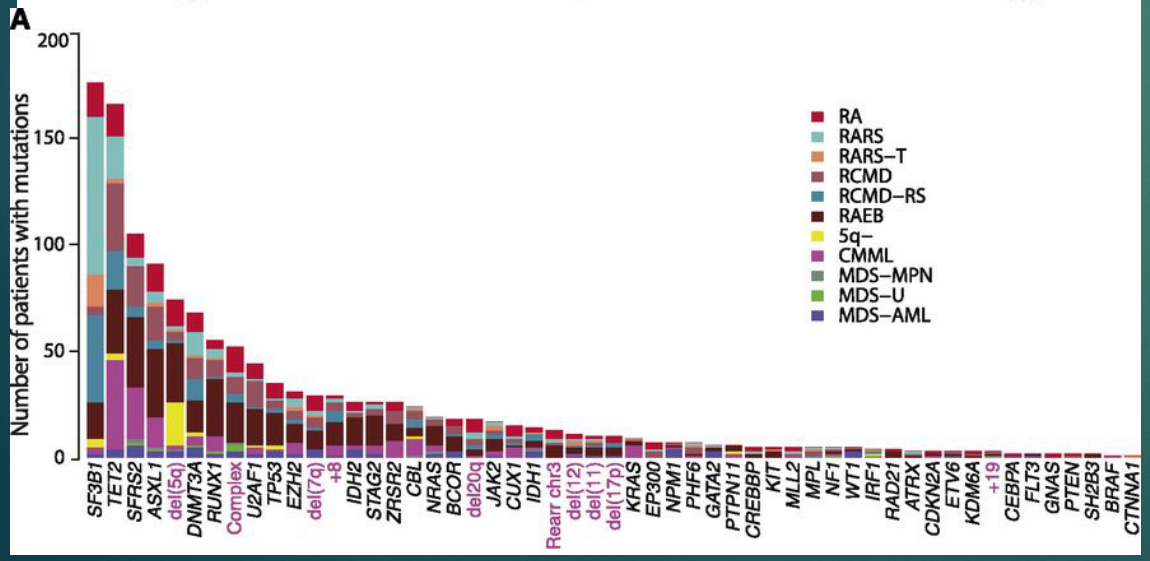
The prevalence of somatic mutations across human cancer types



LB Alexandrov *et al. Nature*, 1-7 (2013) doi:10.1038/nature12477

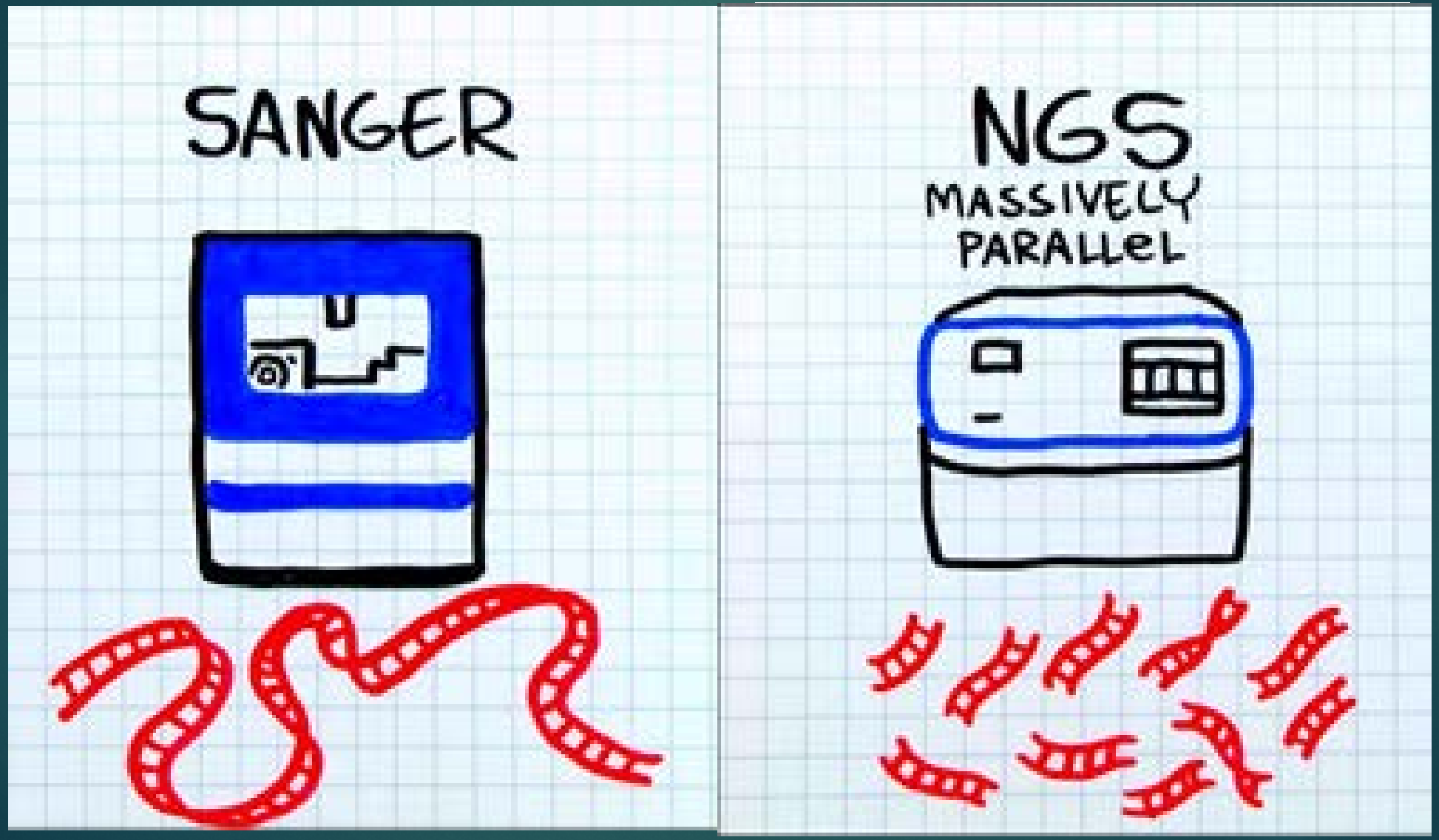


Ley T., et al. NEJM 2013;368:2059-74

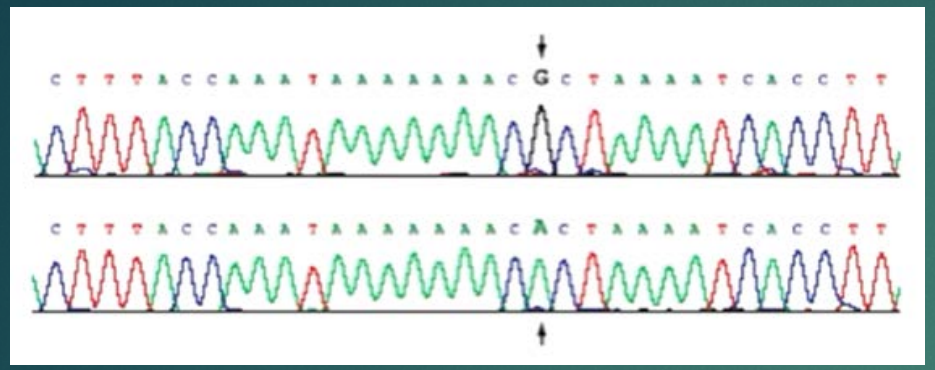


Papaemmanuil E., et al. Blood 2013;122:3616-27

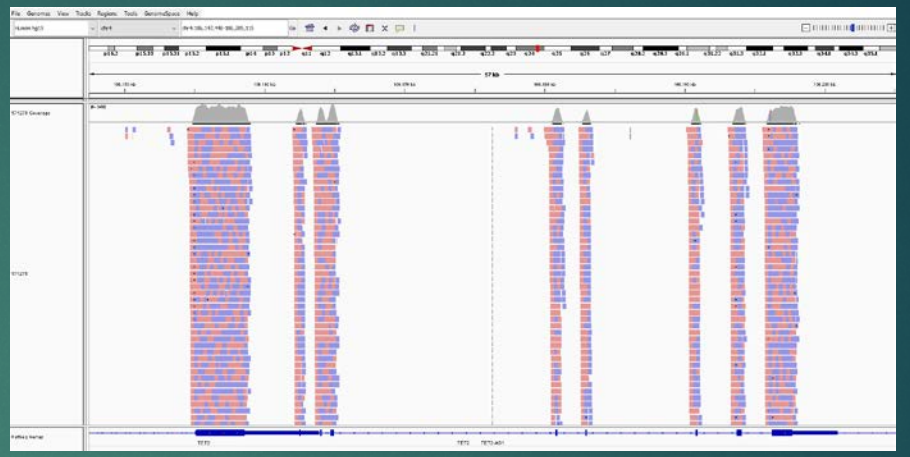
Sanger sequencing - NGS



Sanger sequencing - NGS



- 1 στόχος/δείγμα
- Ευαισθησία 20%
- Περιορισμένο εύρος (500-600bp)
- CNV, αντιμεταθέσεις, μεγάλες ελλείψεις: δεν ανιχνεύονται
- Σύνθετη αλληλουχία: δύσκολο να εκτιμηθεί



- Ευαισθησία ~5-10%
- Εκτεταμένο εύρος (1000's bp)
- CNV, αντιμεταθέσεις, μεγάλες ελλείψεις: δεν ανιχνεύονται καλά
- Σύνθετη αλληλουχία: πιθανά ευκολότερο να εκτιμηθεί

NGS ροή εργασίας

	"Wet Bench" διαδικασία	"Dry Bench" διαδικασία
Προ αναλυτικό στάδιο *Pre-analytic*	Προετοιμασία Βιβλιοθήκης *Library Prep*	• Demultiplexing • Base calling
	Προετοιμασία Μήτρας *Template Prep*	• Στοίχιση, Alignment • Variant calling • Specialized applications
	Αλληλούχιση *Sequencing*	• Variant Annotation (Σχολιασμός) • Variant significance

“Wet Bench” *Pre-analytic*

- Δείγμα είδος (π.χ. φρέσκο vs. FFPE), αντιπηκτικό, κλπ.
- Tumor percentage requirement
  - Need for microdissection (FFPE)
  - Cell sorting
- Εκχύλιση DNA (ή RNA)
- Χρήση φυσιολογικού δείγματος (paired)
  - Στοματικό έκπλυμα, κλπ.

“Wet Bench” *Library Prep*

Amplicon-based

- Highly multiplexed PCR
- Less DNA input required
- Faster library generation
- Limited target region coverage
- CNV assessment more difficult
- Best for hot spot/regions
- Cheaper?

Capture-based

- Bait (oligonucleotide) probe pull-down of fragmented high quality DNA
- More DNA input required
- Longer library preparation
- Better and more even target region coverage
- CNV detection easier

Note: Sample throughput and accuracy similar

“Wet Bench” *Sequencing*

Illumina MiSeq

- Bridge amplification
- Paired end reads possible
- Sequencing-by-synthesis
- Slower (~24 hrs)
- Less artifact
- Higher output*

Ion Torrent S5

- Emulsion droplet PCR
- Single end reads
- Longer read lengths
- Semiconductor chip sequencing
- Homopolymer artifacts
- Faster (<24 hrs)

NGS ροή εργασίας

	"Wet Bench" διαδικασία	"Dry Bench" διαδικασία
Προ αναλυτικό στάδιο *Pre-analytic*	Προετοιμασία Βιβλιοθήκης *Library Prep*	• Demultiplexing • Base calling
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	Αλληλούχιση *Sequencing*	• Variant Annotation (Σχολιασμός) • Variant significance

“Dry Bench” Ανάλυση

- Bioinformatics processing
  - Index demultiplexing, base call and quality, sequencer performance data
  - Secondary pipelines: initial **alignment to reference**, **SNV** and **indel** detection, **variant calling**
  - Additional analyses: breakpoint analysis, structural variant (SV) detection, specialized applications (RNAseq, immunology, microbial, etc.)
  - Data and computationally intensive
- Ερμηνεία (Interpretation)
  - Τελικός σχολιασμός (**variant annotation**) και επιμέλεια της παραλλαγής
  - Καθορισμός Παθογονικότητας (5 level: **pathogenic**, **likely pathogenic**, **variant uncertain significance**, **likely benign**, **benign**)
  - Πιθανή συσχέτιση (συσχετίσεις) με τη γαμετική σειρά
  - Δημιουργία λεπτομερούς αναφοράς

Πολλές πληροφορίες

information overload

“Although not detracting from the excitement and opportunity that the current situation affords us, we would introduce a note of caution. Like modern-day archeologists confronted with ancient Egyptian hieroglyphs, our current ability to confidently translate the presence of these mutations into clinical gains for individual patients significantly lags behind our ability to detect them.”

grimwade d, et al. blood 2016;127(1):29-41

Genomics of AML: Clinical Applications of Next-Generation Sequencing

John S. Welch[1] and Daniel C. Link[1]

[1]Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, MO

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 22, 2012 VOL. 366 NO. 12

Prognostic Relevance of Integrated Genetic Profiling in Acute Myeloid Leukemia

Jay P. Patel, Mithat Gönen, Ph.D., Maria E. Figueroa, M.D., Hugo Fernandez, M.D., Zhuoxin Sun, Ph.D.,

International Journal of Laboratory Hematology

The Official journal of the International Society for Laboratory Hematology

REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes

E. J. DUNCAVAGE, B. TANDON

Pathogenesis 2 (2015) 9–16

Contents lists available at ScienceDirect

Pathogenesis

journal homepage: http://www.pathogenesisjournal.com/

Review article

The impact of next generation sequencing technologies on haematological research – A review

Jessica S. Black [a], Manuel Salto-Tellez [a, b], Ken I. Mills [a], Mark A. Catherwood [a, c, *]

[a] Centre for Cancer Research and Cell Biology (CCRCB), Queen's University Belfast, UK
[b] Tissue Pathology Department, Belfast Health and Social Care Trust, UK
[c] Haematology Department, Belfast Health and Social Care Trust, UK

ARTICLES

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley[1,2,3,4]*, Elaine R. Mardis[2,3]*, Li Ding[2,3], Bob Fulton[3], Michael D. McLellan[3], Ken Chen[3], David Dooling[3],

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 30, 2013 VOL. 368 NO. 22

Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network

Targeted Therapies and Cancer (I)

Disease	Target	Therapies	Notes
Colon Ca	*EGFR*	cetuximab	Contraindicated if K/*NRAS*, *RAF* mutation
	PD1	pembrolizumab	MSI status
NSCLC	*EGFR*	cetuximab	T790M mutation resistance
	RET	cabozantinib	Rare, young age, non-smoker
	ROS1, *ALK*	crizotinib	Rare, female, younger
Other solid Tumors	*PD1*	pembrolizumab	H&N squamous Ca; GU tumors, breast

Targeted Therapies and Cancer (II)

Disease	Target	Therapies	Notes
CHL	*PD1*	pembrolizumab	ABVD Rx effective
NHL	BCR signaling (BTK, PIK3CD)	Ibrutinib, idelasilib	ABC-DLBCL, LPL/WM, CLL
	CD20, CD22, CD25	Rituximab, epratuzumab, alemtuzumab, blinatumomab	Humanized antibodies, BiTE agents, etc.
	BCL2	Venetoclax	
	CD19	CAR-T	ALL, expanding
MM	26S proteasome	Bortezomib	+2nd generation drugs
	CRBN pathway	Lenalidomide (IMiD)	+2nd generation drugs

Targeted Therapies and Cancer (III)

- Μυελογενείς κακοήθειες:
  - *BCR-ABL* (CML, Ph+ B-ALL)
  - *PML-RARA* (APL)
- Λοιπές:
  - όχι διαδεδομένες στοχευμένες θεραπείες

Signal Transduction:

- *JAK2*
- *KIT*
- *FLT3*
- *CSF3R*
- *K/NRAS*
- *MPL*
- *CBL*
- *PTPN11*

RNA Splicing:

- *SF3B1*
- *SRSF2*
- *U2AF1*
- *ZRSR2*

Transcription Factor:

- *GATA1/2*
- *RUNX1*
- *CEBPA*
- *ETV6*
- *NOTCH1*
- *BCOR*
- *WT1*
- *PHF6*

Chromosome stability:

- *TERT*
- Cohesin complex (*STAG1, SMC3, RAD21*)

Tumor Suppressor:

- *TP53*

Epigenetic:

- *IDH1/2*
- *TET2*
- *EZH2*
- *ASXL1*
- *DNMT3A*

Other:

- *SETBP1*
- *CALR*
- *NPM1*

MYELOID NEOPLASIA

Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia

Klaus H. Metzeler,[1-3] Tobias Herold,[1-3] Maja Rothenberg-Thurley,[1] Susanne Amler,[4] Maria C. Sa
Stephanie Schneider,[1] Nikola P. Konstandin,[1] Annika Dufour,[1] Kathrin Bräundl,[1-3] Bianka Ksienz
Luise Hartmann,[1-3] Philipp A. Greif,[1-3] Michael Fiegl,[1] Marion Subklewe,[1-3] Stefan K. Bohlander,
Andreas Faldum,[4] Wolfgang E. Berdel,[7] Bernhard Wörmann,[8] Thomas Büchner,[7] Wolfgang Hi
and Karsten Spiekermann,[1-3] on behalf of the AMLCG Study Group

Blood. 2016;128(5):686-698

The NEW ENGLAND JOURNAL of MEDICINE

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Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network

Bacher et al. *Blood Cancer Journal* (2018)8:113
DOI 10.1038/s41408-018-0148-6

Blood C

REVIEW ARTICLE

Challenges in the introduc generation sequencing (N diagnostics of myeloid malignancies into clinical routine use

Ulrike Bacher[1,2], Evgenii Shumilov[3], Johanna Flach[4], Naomi Porret[1], Raphael Joncourt[1], Gertrud Wiedemann[1], Martin Fiedler[2], Urban Novak[5], Ursula Amstutz[2] and Thomas Pabst[5]

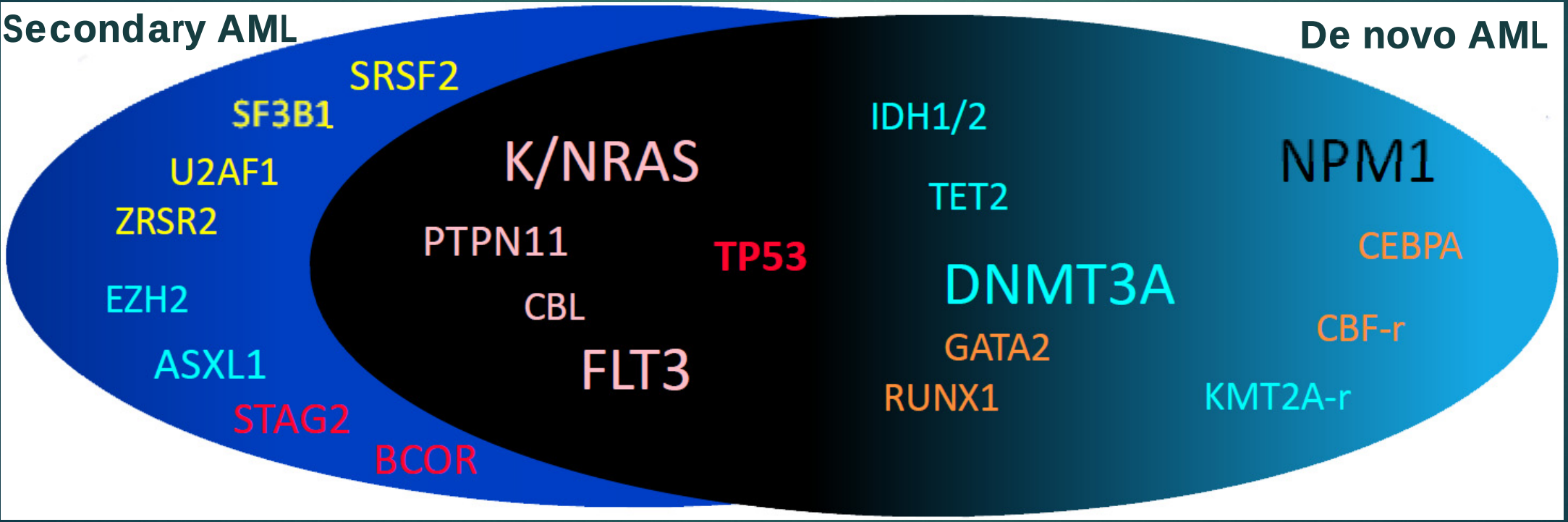
MYELOID NEOPLASIA

Acute myeloid leukemia ontogeny is defined by distinct somatic mutations

R. Coleman Lindsley,[1] Brenton G. Mar,[2] Emanuele Mazzola,[3] Peter V. Grauman,[4] Sarah Shareef,[4] Steven L. Allen,[5] Arnaud Pigneux,[6] Meir Wetzler,[7] Robert K. Stuart,[8] Harry P. Erba,[9] Lloyd E. Damon,[10] Bayard L. Powell,[11] Neal Lindeman,[12] David P. Steensma,[1] Martha Wadleigh,[1] Daniel J. DeAngelo,[1] Donna Neuberg,[3] Richard M. Stone,[1] and Benjamin L. Ebert[4]

Blood. 2015 Feb 26;125(9):1367-76

Μοτίβα μεταλλάξεων στην ΟΜΛ

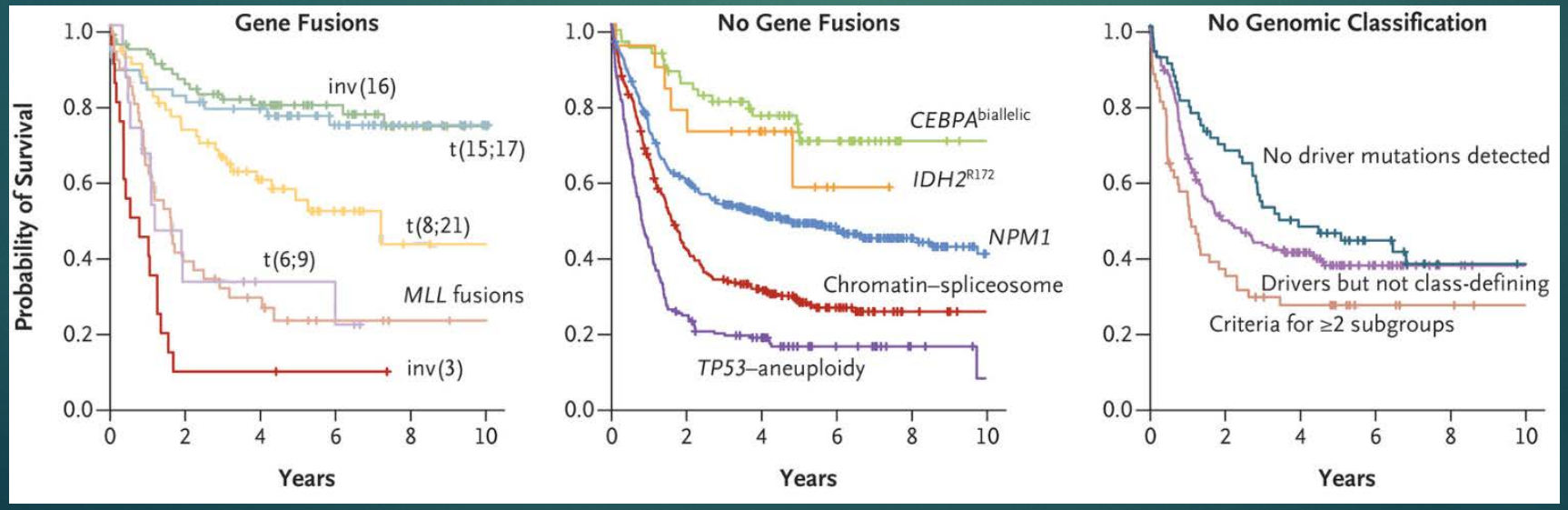


Blood. 2015;125(9):1367-1376

Genomic Classification and Prognosis in Acute Myeloid Leukemia

Elli Papaemmanuil, et al.

N Engl J Med 2016; 374:2209-2221 DOI: 10.1056/NEJMoa1516192



Initial Diagnostic Workup of Acute Leukem

Guideline From the College of American Pathologists and the Americ of Hematology

Daniel A. Arber, MD; Michael J. Borowitz, MD, PhD; Melissa Cessna, MD; Joan Etzell, MD; Kathryn F
Robert P. Hasserjian, MD; J. Douglas Rizzo, MD; Karl Theil, MD; Sa A. Wang, MD; Anthony T. Smith, MLS; R. Br
Nicole E. Thomas, MPH, CT(ASCP)cm; James W. Vardiman, MD

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

Hartmut Döhner,[1] Elihu Estey,[2] David Grimwade,[3] Sergio Amadori,[4] Frederick R. Appelbaum,[2] Thom
Hervé Dombret,[6] Benjamin L. Ebert,[7] Pierre Fenaux,[8] Richard A. Larson,[9] Ross L. Levine,[10] Frances
Tomoki Naoe,[11] Dietger Niederwieser,[12] Gert J. Ossenkoppele,[13] Miguel Sanz,[14] Jorge Sierra,[15] Mar
Hwei-Fang Tien,[16] Andrew H. Wei,[17,18] Bob Löwenberg,[19] and Clara D. Bloomfield[20]

National Comprehensive Cancer Network®

NCCN

Acute Myeloid Leukemia, Version 3.2017

Clinical Practice Guidelines in Oncology

Margaret R. O'Donnell, MD; Martin S. Tallman, MD;
Camille N. Abboud, MD; Jessica K. Altman, MD;
Frederick R. Appelbaum, MD; Daniel A. Arber, MD;
Vijaya Bhatt, MD; Dale Bixby, MD, PhD; William Blum, MD;
Steven E. Coutre, MD; Marcos De Lima, MD;
Amir T. Fathi, MD; Melanie Fiorella, MD; James M. Foran, MD;
Steven D. Gore, MD; Aric C. Hall, MD; Patricia Kropf, MD;
Jeffrey Lancet, MD; Lori J. Maness, MD; Guido Marcucci, MD;

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

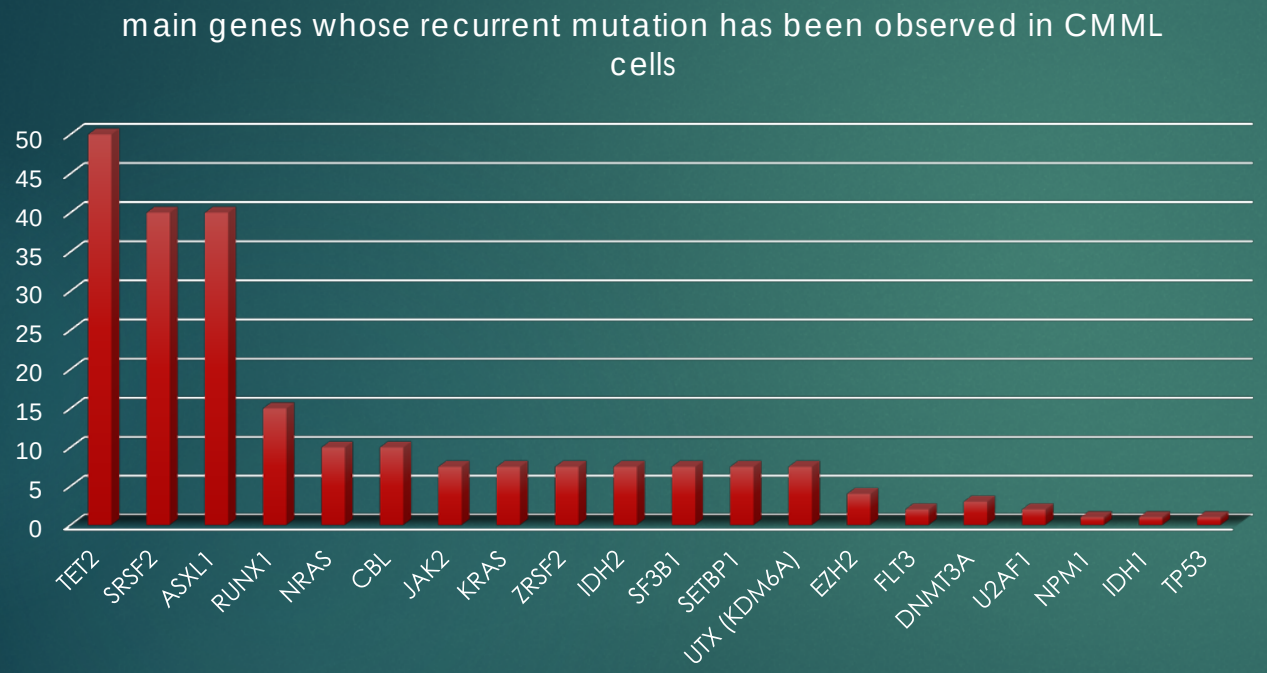
The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

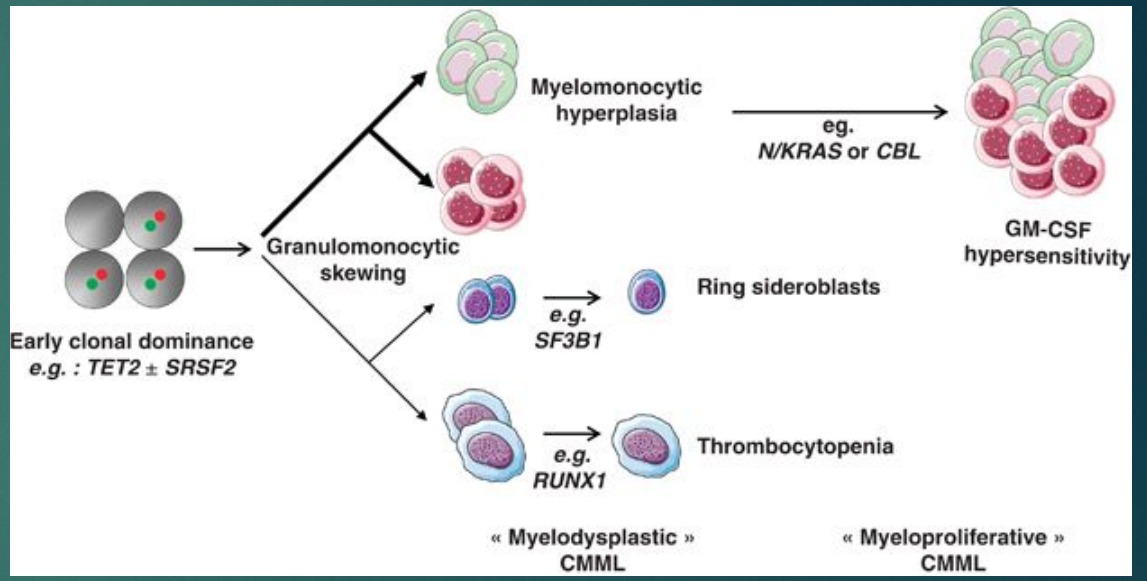
Daniel A. Arber,[1] Attilio Orazi,[2] Robert Hasserjian,[3] Jürgen Thiele,[4] Michael J. Borowitz,[5] Michelle M. Le Beau,[6]
Clara D. Bloomfield,[7] Mario Cazzola,[8] and James W. Vardiman[9]

Προφίλ μεταλλάξεων γονιδίων: Μυελογενείς κακοήθειες

AML:	
Tier 1	*FLT3*-ITD, *IDH2*, *TP53*, *CEBPA*-dm
Tier 2	Spliceosome/epigenetic, *TP53*, *KIT*, *RUNX1*,*?DNMT3A*
Tier 3	Σχετίζονται με γαμετική σειρά: *GATA2*, *DDX41*, *ANKRD26*, *CEBPA*...
MDS:	*SF3B1*, *TP53*, άλλο (φορτίο μετάλλαξης)
MDS/MPN:	*ASXL1, TET2, SRSF2, JAK2, SETBP1, ETNK1, RAS* μονοπάτι
MPN (PMF):	*JAK2, CALR, MPL, ASXL1, TP53, CSF3R*

CMML:





An evolutionary perspective on chronic myelomonocytic leukemia
Itzykson R and Solary E., Leukemia 2013;27:1441

Clinical Implications of Clonal Hematopoiesis

David P. Steensma, MD

Mayo Clin Proc. 2018;93(8):1122-1130

ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoum, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svantesson, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönberg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.

Nat Med. 2014 December ; 20(12): 1472–1478. doi:10.1038/nm.3733.

Age-related cancer mutations associated with clonal hematopoietic expansion

Mingchao Xie[1,2,*], Charles Lu[1,*], Jiayin Wang[1,2,*], Michael D. McLellan[1], Kimberly J. Johnson[3], Michael C. Wendl[1,4,5], Joshua F. McMichael[1], Heather K. Schmidt[1], Venkata Yellapantula[1,2], Christopher A. Miller[1], Bradley A. Ozenberger[1,2], John S. Welch[2,6], Daniel C. Link[2,6], Matthew J. Walter[2,6], Elaine R. Mardis[1,2,4,6], John F. Dipersio[2,6], Feng Chen[2,6], Richard K. Wilson[1,2,4,6], Timothy J. Ley[1,2,4,6], and Li Ding[1,2,4,6,#]

ARTICLE

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DOI: 10.1038/ncomms12484 OPEN

Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults

Andrew L. Young[1,2], Grant A. Challen[3], Brenda M. Birmann[4] & Todd E. Druley[1,2]

Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes

BLOOD, 2 JULY 2015 • VOLUME 126, NUMBER 1

David P. Steensma,[1] Rafael Bejar,[2] Siddhartha Jaiswal,[3] R. Coleman Lindsley,[1] Mikkael A. Sekeres,[4] Robert P. Hasserjian,[5] and Benjamin L. Ebert[3]

[1]Department of Medical Oncology, Division of Hematological Malignancies, Dana-Farber Cancer Institute and Brigham and Women's Hospital, Boston, MA; [2]Division of Hematology-Oncology, Moores Cancer Center at the University of California at San Diego, La Jolla, CA; [3]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [4]Department of Hematologic Oncology and Blood Disorders, Cleveland Clinic Taussig Cancer Institute, Cleveland, OH; and [5]Department of Pathology, Massachusetts General Hospital, Boston, MA

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

Siddhartha Jaiswal, M.D., Ph.D., Pierre Fontanillas, Ph.D., Jason Flannick, Ph.D., Alisa Manning, Ph.D., Peter V. Grauman, B.A., Brenton G. Mar, M.D., Ph.D., R. Coleman Lindsley, M.D., Ph.D., Craig H. Mermel, M.D., Ph.D., Noel Burtt, B.S., Alejandro Chavez, M.D., Ph.D., John M. Higgins, M.D., Vladislav Moltchanov, Ph.D., Frank C. Kuo, M.D., Ph.D., Michael J. Kluk, M.D., Ph.D., Brian Henderson, M.D., Leena Kinnunen M.Sc., Heikki A. Koistinen, M.D., Ph.D., Claes Ladenvall, Ph.D., Gad Getz, Ph.D., Adolfo Correa, M.D., Ph.D., Benjamin F. Banahan, Ph.D., Stacey Gabriel, Ph.D., Sekar Kathiresan, M.D., Heather M. Stringham, Ph.D., Mark I. McCarthy, M.D.,* Michael Boehnke, Ph.D.,* Jaakko Tuomilehto, M.D., Ph.D., Christopher Haiman, Sc.D., Leif Groop, M.D., Ph.D., Gil Atzmon, Ph.D., James G. Wilson, M.D., Donna Neuberg, Sc.D., David Altshuler, M.D., Ph.D.,* and Benjamin L. Ebert, M.D., Ph.D.†

Κλωνική αίμοποίηση
Clonal Hematopoiesis, CH

η επέκταση των αιμοκυττάρων που προέρχονται από ένα μόνο προγονικό αιμοποιητικό κύτταρο και είναι το καθοριστικό χαρακτηριστικό των αιματολογικών καρκίνων

CH είναι μία συχνή συνέπεια της γήρανσης

Κλωνική αίμοποίηση
Clonal Hematopoiesis, CH

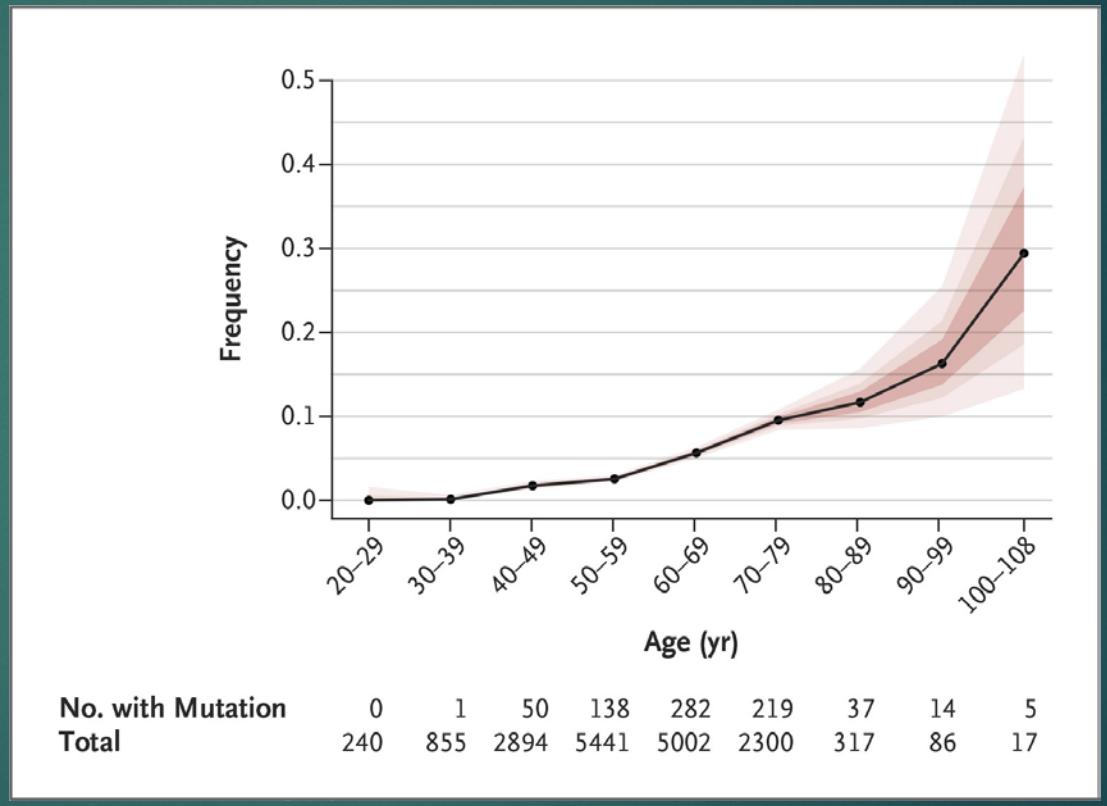

Figure 1. Prevalence of Somatic Mutations, According to Age.

Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

CHIP-ICUS-CCUS-Cancer Spectrum

CHIP	• Ασθενείς χωρίς προηγούμενη ή ταυτόχρονη αιματολογική κακοήθεια • Σωματικές μεταλλάξεις σε ένα ή μερικές φορές >1 γονίδιο σε ποσοστό >2% variant allele fraction (VAF) • *TET2*, *DNMT3A*, *ASXL1*, *SF3B1*, *TP53* • Αύξηση επίπτωσης με την ηλικία • Αναγνωρίστηκαν με WES/WGS σε μεγάλους πληθυσμούς • Κίνδυνος εξέλιξης σε μυελογενή κακοήθεια (0.5-1% /έτος; 10X κινδύνου)
ICUS	• Ανεξήγητη κυτταροπενία με απουσία κυτταρογενετικού και ανιχνεύσιμου με NGS κλωνικού πληθυσμού • Αβέβαιος κίνδυνος νεοπλασματικής εξέλιξης, πιθανά χαμηλός
CCUS	CHIP + ICUS • Ο κίνδυνος εξέλιξης σε μυελογενή κακοήθεια είναι υψηλότερος

CHIP: Associations

- CHIP is highly prevalent (>90%) after age 50 with high sensitivity techniques
- As defined, CHIP increases risk for developing a hematologic neoplasm
  - Risk increases if multiple mutations, higher VAF%
- Risk of all-cause mortality and cardiovascular disease
- Therapy-related myeloid neoplasms: higher risk for development of T-MN with pre-existing CHIP
- Presence of CHIP in donor HSCT allograft can result in impaired graft function in recipient but not donor-derived leukemia
- Not all CHIP is equal
  - *TET2* and *DNMT3A* very common, yet not clearly associated with adverse effects on hematopoietic function, or risk of myeloid neoplasia
  - Clonal size expands with age in some individuals: ? Compensation for failing wild type HSCs

Nat Commun. 2016;7:12484, Lancet Oncol. 2017;18:100–11, Lancet Oncol. 2017;18:112–21, Blood, 2017;130:91-94, Blood, 2017;130(6):753-762

CCUS: κίνδυνος νεοπλασματικής εξέλιξης

- Earlier studies: highly suggestive of increased risk for ICUS with clonal hematopoietic mutations to progress to overt myeloid cancers
- Recent large studies confirm and delineate the risk more specifically
  - Longitudinal follow-up
  - VAF>10%; >2 mutations and spliceosome mutations with classic CHIP alterations have very high PPV for progression to myeloid neoplasm
    - HR for progression 13.9
- Certain mutation patterns may be more strongly predictive and can provide presumptive evidence for early myeloid neoplasia in the absence of definitive morphologic criteria or cytogenetic abnormalities
- Conversely the absence of mutations (with sufficiently comprehensive screening) has very high NPV for neoplasia, even in the setting of morphologic “atypia”

Blood. 2015;126(21):2362-2365, Blood. 2015;126(21):2355-2361, Blood. 2017;129(25):3371-3378, Am. J. Hematol. 91:1234–1238, 2016

NGS και Myeloma

Γενετικές κατηγορίες	κίνδυνος
Hyperdiploid/CNA	
HD (odd chromosome #'s)	Standard (35-40%)
CNA at +1q, del1p, del17p	High (5-10% each)
Translocations	
t(11;14) *CCND1*, t(6;14) *CCND3*	Standard (15%)
t(14;16) και t(14;20) *MAF;MAFB*	High (<5%)
t(4;14) *FGFR3*; NSD2 (*MMSET, WHSC1*)	Intermediate (10-15%)
8q24 *MYC*	High (20%)

Notes:

- Based on Mayo mSMART 2.0
- High risk GEP signature is also high risk feature

NGS και Myeloma

- Modify or additionally stratify current FISH-defined risk classification
- Focus on additional prognostic alterations and therapeutic/resistance genotypes
- “Ideal state” is a single test to capture most relevant alterations in SMM, MM (e.g. SNV, SV, CNV)
  - Technically difficult
- Possible utility for refractory/relapsed disease
- In early stages of application and clinical utility determination (indications)

NGS and Myeloma

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NGS and Myeloma

MAPK

- *N/KRAS*
- *BRAF*

IMiD

- *CRBN*
- *CUL4B*
- *IKZF1*
- *IRF4*

Other

- *FGFR3*
- *CCND1*
- *FAM46C*
- *DIS3*

NFKB

- *TRAF3*
- *CYLD*

DNA DR:

- *TP53*
- *ATM*
- *ATR*
- *EGR1*

Ενδείξεις για εφαρμογή NGS

Χρήσιμο

- Διάγνωση ΟΜΛ
- Υποτροπή ΟΜΛ
- Διάγνωση Dx MDS
- MDS/MPN (π.χ. CMML)
- MPN-PMF ή τριπλά αρνητικά
- Ανεξήγητη κυτταροπενία (“ICUS”)

Δεν ενδείκνυται/Αβέβαιο

- Palliative situation
- Μαστοκυττάρωση (*KIT*D816V, *KIT* seq)
- MPN-PV, MPN-ET (MPN-R)
- MPN-CNL (*CSF3R*)
- CML (*BCR-ABL*)
- CEL/HES και άλλες Νεοπλασματικές Ηωσινοφιλίες (FISH)

Σύνοψη

- NGS είναι μία προσιτή πλέον τεχνολογία για εφαρμογή στα κλινικά εργαστήρια
- με κόστος που συνεχώς μειώνεται
- με μεγάλο όγκο πληροφοριών που πρέπει να διαχειριστούν

Βρίσκει εφαρμογή

- στη **διάγνωση** των αιματολογικών κακοηθειών
- στην **πρόγνωση**,
  - στη **θεραγνωστική**
- κατανόηση: co-mutation patterns + clonal hematopoiesis

Σας ευχαριστώ