

# Η προσέγγιση του ασθενή με λευχαιμία την εποχή του NGS

**Μαρία Γαροφαλάκη**

Βιολόγος, Εργαστήριο Μοριακής Βιολογίας,  
Αιματολογική-Λεμφωμάτων Κλινική και ΜΜΜΟ

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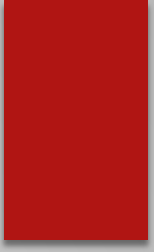
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Intuition in Healthcare



Rontis  
Driven by innovation



Specifar  
A Teva Company



«αλληλούχιση επόμενης γενιάς»

**N**ext **G**eneration **S**equencing, **NGS**,

αναφέρεται στις τεχνολογίες μαζικής και παράλληλης αλληλούχισης που επιτρέπουν την ταχεία και αξιόπιστη αλληλούχιση μεγάλου αριθμού γονιδίων ταυτόχρονα.

# Νέο εργαλείο για διαγνωστική & έρευνα



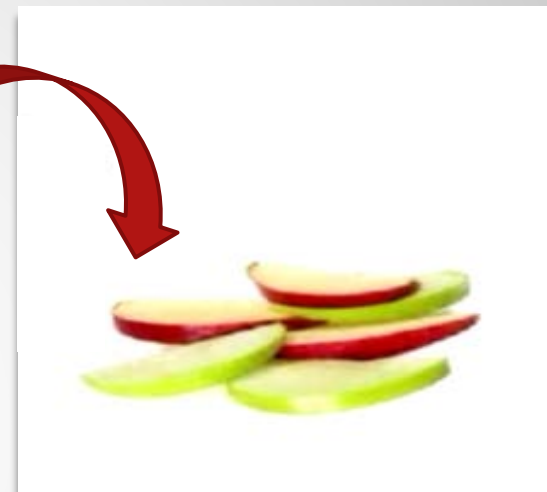
**Whole Genome Seq, WGS**

3 000 000 000 pb



**Whole Exome Seq, WES**

21 000 genes

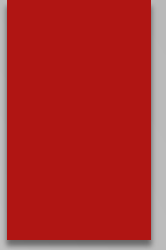


**Targeted sequencing, panels**

100 genes

Gene panels: χρησιμοποιούνται στα κλινικά εργαστήρια

# NGS



Targeted panels

WES

WGS

**RNASeq**

**Immune Seq**

**ChIP Seq**

Methylome Seq



ELSEVIER

Review article

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

Jessica S. Black<sup>a</sup>, Manuel Salto-Tellez<sup>a, b</sup>, Ken I. Mills<sup>a</sup>, Mark A. Catherwood<sup>a, c, \*</sup>

<sup>a</sup> Centre for Cancer Research and Cell Biology (CCRCB), Queen's University Belfast, UK

<sup>b</sup> Tissue Pathology Department, Belfast Health and Social Care Trust, UK

<sup>c</sup> Haematology Department, Belfast Health and Social Care Trust, UK

## ARTICLES

## DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley<sup>1,2,3,4\*</sup>, Elaine R. Mardis<sup>2,3\*</sup>, Li Ding<sup>2,3</sup>, Bob Fulton<sup>3</sup>, Michael D. McLellan<sup>3</sup>, Ken Chen<sup>3</sup>, David Dooling<sup>3</sup>

## 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



## Genomics of AML: Clinical Applications of Next-Generation Sequencing

John S. Welch<sup>1</sup> and Daniel C. Link<sup>1</sup>

<sup>1</sup>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

| Pathway                 | Syndrome                                                                                                     | Gene           | Pathway                 | Syndrome                      | Gene                |
|-------------------------|--------------------------------------------------------------------------------------------------------------|----------------|-------------------------|-------------------------------|---------------------|
| Transcription factor    | Familial platelet disorder with propensity to myeloid malignancy<br>Familial AML<br>GATA2-spectrum disorders | <i>RUNX1</i>   | Telomere maintenance    | Dyskeratosis congenita        | <i>DKC1</i>         |
|                         |                                                                                                              |                |                         |                               | <i>TERC</i>         |
|                         |                                                                                                              |                |                         |                               | <i>TERT</i>         |
|                         |                                                                                                              |                |                         |                               | <i>NOLA3/NOP10</i>  |
|                         |                                                                                                              |                |                         |                               | <i>NOLA2/NHP2</i>   |
|                         | Susceptibility to ALL 3<br>Thrombocytopenia 5                                                                | <i>CEBPa</i>   | Checkpoint regulation   | Li Fraumeni                   | <i>TINF2</i>        |
|                         |                                                                                                              | <i>GATA2</i>   |                         |                               | <i>WRAP53/TCAB1</i> |
|                         |                                                                                                              |                |                         |                               | <i>CTC1</i>         |
|                         |                                                                                                              |                |                         |                               | <i>RTEL1</i>        |
|                         |                                                                                                              |                |                         |                               | <i>ACD/TPP1</i>     |
|                         |                                                                                                              |                |                         |                               | <i>PARN</i>         |
|                         |                                                                                                              |                |                         |                               | <i>NAF1</i>         |
|                         |                                                                                                              |                |                         |                               | <i>STN1</i>         |
|                         |                                                                                                              | <i>PAX5</i>    |                         |                               | <i>TP53</i>         |
|                         |                                                                                                              | <i>ETV6</i>    |                         |                               |                     |
| Ribosomal abnormalities | Diamond Blackfan Anemia                                                                                      | <i>GATA1</i>   | RAS pathway             | Noonan syndrome               | <i>PTPN11</i>       |
|                         |                                                                                                              | <i>RPL5</i>    |                         |                               |                     |
|                         |                                                                                                              | <i>RPL11</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPL15</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPL23</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPL26</i>   | Other                   | Severe congenital neutropenia | <i>CBL</i>          |
|                         |                                                                                                              | <i>RPL27</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPL31</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPL35a</i>  |                         |                               |                     |
|                         |                                                                                                              | <i>RPL36</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS7</i>    |                         |                               |                     |
|                         |                                                                                                              | <i>RPS10</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS15</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS17</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS19</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS24</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS26</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS27</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS27A</i>  |                         |                               |                     |
|                         |                                                                                                              | <i>RPS28</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>RPS29</i>   |                         |                               |                     |
|                         |                                                                                                              | <i>(TSR2)†</i> |                         |                               |                     |
|                         |                                                                                                              | <i>SBDS</i>    |                         |                               |                     |
|                         | Shwachman Diamond Syndrome                                                                                   |                | Chromosomal abnormality | Down's syndrome               | <i>ELANE</i>        |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | Thrombocytopenia 2            | <i>ANKRD26</i>      |
|                         |                                                                                                              |                |                         |                               | <i>WAS</i>          |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | X-linked neutropenia          | <i>WAS</i>          |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | Wiskott Adrich Syndrome       | <i>HAX1</i>         |
|                         |                                                                                                              |                |                         |                               | <i>DDX41</i>        |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | SCN3                          | <i>SAMD9L</i>       |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | Ataxia Pancytopenia Syndrome  | <i>SRP72</i>        |
|                         |                                                                                                              |                |                         |                               | <i>NF1</i>          |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | Neurofibromatosis             |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         | Trisomy 2                     |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |
|                         |                                                                                                              |                |                         |                               |                     |

## Germline Genetic Predisposition to Hematologic Malignancy

Elissa Furutani and Akiko Shimamura



July 2016 Volume 16, Issue 7, Pages 417–428.

## Evaluation of Patients and Families With Concern for Predispositions to Hematologic Malignancies Within the Hereditary Hematologic Malignancy Clinic (HHMC)

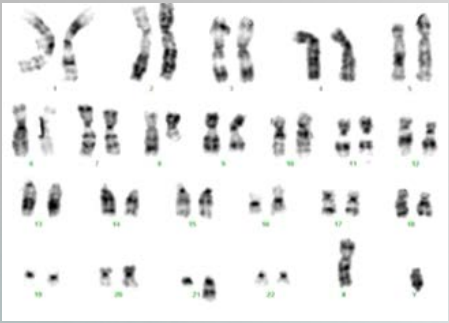
Courtney D. DiNardo,<sup>1</sup> Sarah A. Bannon,<sup>2</sup> Mark Routbort,<sup>3</sup> Anna Franklin,<sup>4</sup> Maureen Mork,<sup>2</sup> Mary Armanios,<sup>5</sup> Emily M. Mace,<sup>6</sup> Jordan S. Orange,<sup>6</sup> Meselle Jeff-Eke,<sup>7</sup> Jane E. Churpek,<sup>7</sup> Koichi Takahashi,<sup>1</sup> Jeffrey L. Jorgensen,<sup>3</sup> Guillermo Garcia-Manero,<sup>1</sup> Steve Kornblau,<sup>1</sup> Alison Bertuch,<sup>8</sup> Hannah Cheung,<sup>9</sup> Kapil Bhalla,<sup>1</sup> Andrew Futreal,<sup>9</sup> Lucy A. Godley,<sup>7</sup> Keyur P. Patel<sup>3</sup>

## PRECISION HEMATOLOGY

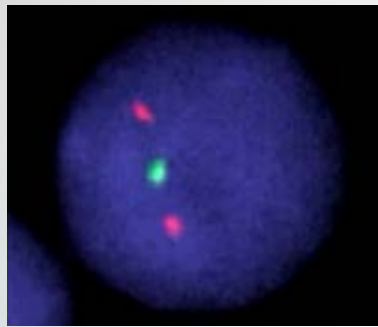
## Genetic predisposition to hematologic malignancies: management and surveillance

Lucy A. Godley<sup>1,2</sup> and Akiko Shimamura<sup>3</sup>*Blood.* 2017;130(4):424-432

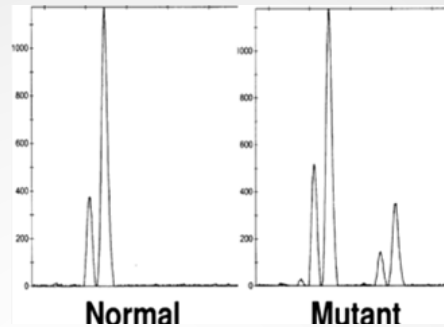
# Εξέλιξη της γενετικής διαγνωστικής



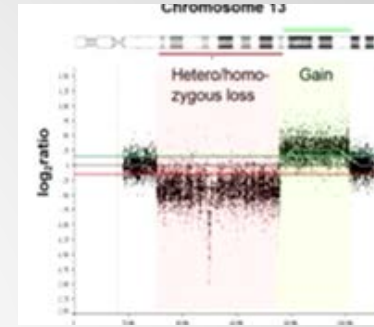
▶ G-Banding



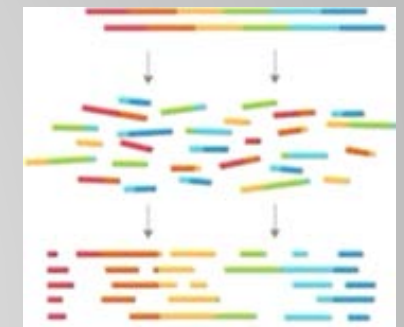
▶ FISH



▶ PCR



▶ microarrays

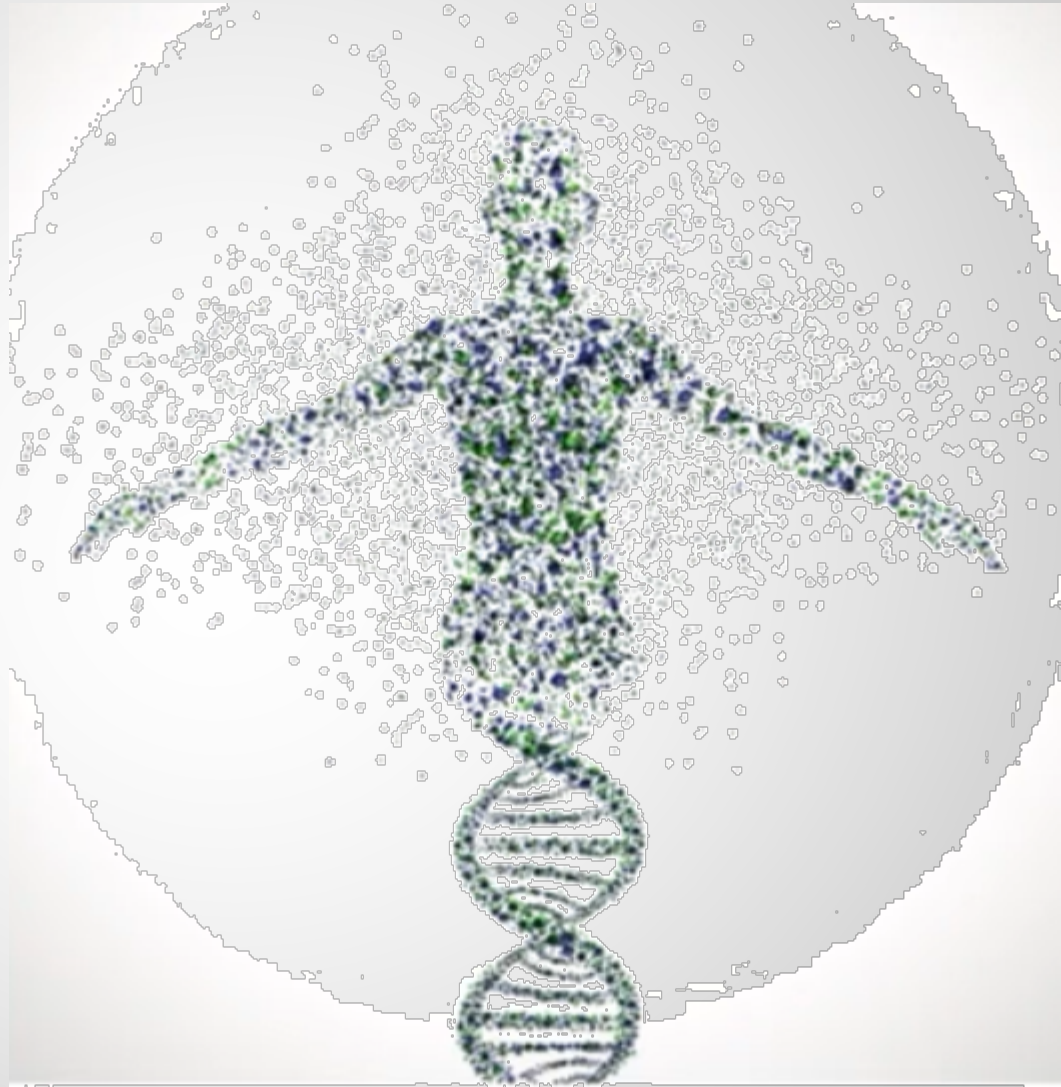


▶ NGS

1970

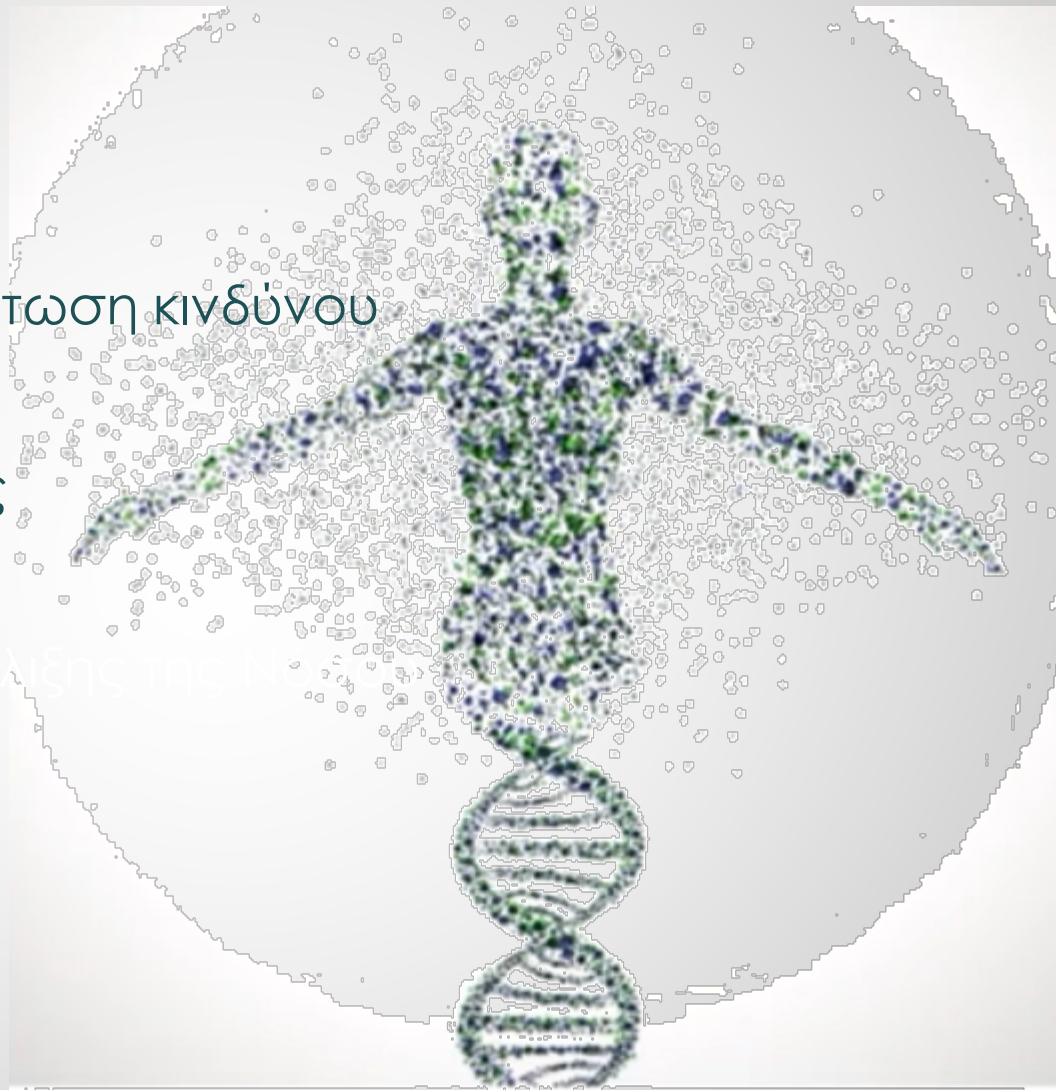
2010

# Γιατί γενετική διάγνωση στην αιματολογία?



# Γιατί γενετική διάγνωση στην αιματολογία?

- ▶ Διάγνωση
- ▶ Πρόγνωση – διαστρωμάτωση κινδύνου
- ▶ Θεραπευτικές επιπτώσεις
- ▶ Παρακολούθηση της εξέλιξης της νόσου
- ▶ Ανίχνευση EYN



# Γιατί γενετική διάγνωση στην αιματολογία?

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





Review

# Minimal Residual Disease Monitoring with Next-Generation Sequencing Methodologies in Hematological Malignancies

Ricardo Sánchez <sup>1,2,\*</sup> , Rosa Ayala <sup>1,2,3,4</sup> and Joaquín Martínez-López <sup>1,2,3,4,\*</sup>

MYELOID NEOPLASIA | OCTOBER 18, 2018

**Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML**

*Blood* (2018) 132 (16): 1703–1713.

ARTICLE

Acute Myeloid Leukemia



Ferrata Storti Foundation

## A novel deep targeted sequencing method for minimal residual disease monitoring in acute myeloid leukemia

Esther Onecha,<sup>1,2</sup> Maria Linares,<sup>1,2</sup> Inmaculada Rapado,<sup>1,2,3</sup>  
Yanira Ruiz-Heredia,<sup>1,2</sup> Pilar Martinez-Sanchez,<sup>1</sup> Teresa Cedena,<sup>1,2,3,4</sup>  
Marta Pratcorona,<sup>5</sup> Jaime Perez Oteyza,<sup>6</sup> Pilar Herrera,<sup>7</sup> Eva Barragan,<sup>4,8</sup>  
Pau Montesinos,<sup>4,8</sup> Jose Antonio Garcia Vela,<sup>9</sup> Elena Magro,<sup>10</sup>  
Eduardo Anguita,<sup>11</sup> Angela Figuera,<sup>12</sup> Rosalia Rianza,<sup>13</sup>  
Pilar Martinez-Barranco,<sup>14</sup> Beatriz Sanchez-Vega,<sup>1,2</sup> Josep Nomdedeu,<sup>5</sup>  
Miguel Gallardo,<sup>2\*</sup> Joaquin Martinez-Lopez<sup>1,2,3,4\*</sup> and Rosa Ayala<sup>1,2,3,4\*</sup>

# TruSight™ Myeloid Sequencing Panel

Using expert-defined content and proven next-generation sequencing (NGS) technology to identify somatic mutations in hematologic malignancies.

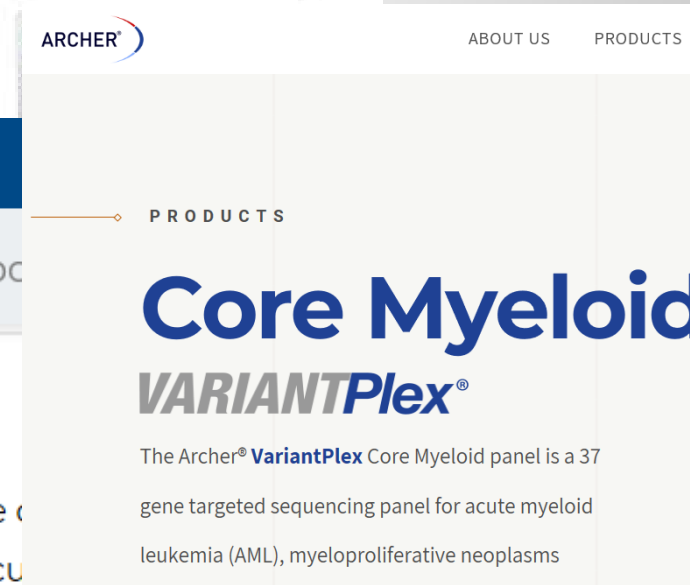
## MYELOID SOLUTION™ BY SOPHiA GENETICS

The Myeloid Solution (MYS) by SOPHiA GENETICS is a genomic application that bundles a smart capture-based target enrichment kit with the analytical power of SOPHiA™ AI and full access to SOPHiA DDM® platform.



### AML Geneprofile Panel®

Acute Myeloid Leukemia (AML) is a heterogeneous disorder characterised by the presence of abnormal cells in the bone marrow and peripheral blood. The disorder arises from a series of recurrent



# VariantPlex AML Focus

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11 Genes

Illumina

Highlights

ASXL1, CEBPA, DNMT3A, 750K Recommended Read Depth  
FLT3, IDH1, IDH2, JAK2,  
Panel, NPM1, RUNX1,  
TP53

- ✓ Robust FLT3-ITD detection
- ✓ Deep, full coverage of CEBPA



## FusionPlex™ RNA Kits

- Acute Lymphoblastic Leukemia Kit
- Core Acute Myeloid Leukemia Kit
- Lymphoma Kit
- Myeloid Kit



CD Genomics  
DISEASE PANEL

Home

Custom NGS Panel

Predesigned NGS Panel

Non-NGS Panel

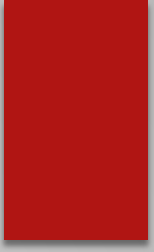
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### SERVICES

|                                |                                                                                                                                                                                   |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| • Custom AML Panel             | This panel contains 42 genes associated with acute myeloid leukemia, including <i>AML1</i> , <i>AML2</i> , <i>AML3</i> and other related genes.                                   |
| • Custom CLL Panel             | The panel contains 28 chronic lymphocytic leukemia-related genes for your selection, such as <i>NOTCH1</i> and <i>SF3B1</i> .                                                     |
| • Custom Core MPN Panel        | This panel collects 34 core genes, such as <i>JAK2V617F</i> , which are associated with myeloproliferative neoplasms for you to choose.                                           |
| • Custom MPN/MDS Overlap Panel | This panel contains 28 genes for you to choose, including <i>RUNX1</i> , <i>CEBPA</i> and others, which are associated with myelodysplastic/myeloproliferative overlap syndromes. |
| • Custom Myeloid Panel         | This panel contains 59 myeloid-related genes for you to choose, such as <i>ASXL1</i> .                                                                                            |

- 
- ▶ Απλοί νουκλεοτιδικοί πολυμορφισμοί

Single nucleotide variants, SNV

- ▶ Μικρές εισδοχές / απαλοιφές

insertions / deletions

- ▶ Ποικιλομορφίες αριθμού αντιγράφων

Copy number variations, CNV

- ▶ Σύνθετες αναδιατάξεις

- ▶ Υβριδικά γονίδια / αντιμεταθέσεις

- ▶ Διάγνωση

- ▶ Πρόγνωση

- ▶ Θεραπεία και παρακολούθηση

# Διάγνωση

May-June 2016, Volume 13, Issue 3

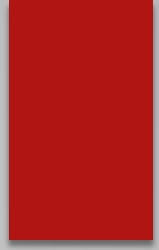
## Next-Generation Sequencing Multi-Gene Mutation Panels in Myeloid Malignancies



THE Hematologist  
ASH NEWS AND REPORTS®

| Actionable Categories | Mutated Genes                                                                                                                                                                       | Clinical Significance                                                                      |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Diagnostic            | "MDS" genes: <i>TET2</i> , <i>DNMT3A</i> , <i>TP53</i> , <i>SF3B1</i> , <i>SRSF2</i> , <i>U2AF1</i> , <i>ZRSR2</i> , <i>ASXL1</i> , <i>RUNX1</i> , <i>EZH2</i> , <i>NRAS</i> , etc. | Markers of clonal hematopoiesis: to distinguish MDS from other benign causes of cytopenias |
|                       | <i>CSF3R</i>                                                                                                                                                                        | Diagnostic marker for chronic neutrophilic leukemia (and more rarely atypical CML)         |
|                       | <i>MYD88</i> L265P                                                                                                                                                                  | Diagnostic marker for lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia             |
|                       | <i>BRAF</i> V600E                                                                                                                                                                   | Diagnostic marker for hairy cell leukemia                                                  |
|                       | <i>JAK2</i> (V617F or exon 12), <i>CALR</i> (exon 9), <i>MPL</i>                                                                                                                    | Distinguish clonal myeloproliferative neoplasm from benign mimics                          |

# πρόγνωση

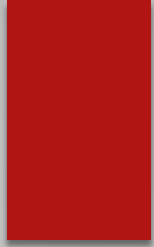


|                                                                               |                    |                                                                      |
|-------------------------------------------------------------------------------|--------------------|----------------------------------------------------------------------|
| <div>Prognostic</div> <div><div>TET2, IDH1/2</div><div>EZH2, NRAS</div></div> | <i>NPM1, CEBPA</i> | Favorable risk in AML<br>(without a <i>FLT3</i> mutation)            |
|                                                                               | <i>RUNX1</i>       | Independently associated<br>with a poor prognosis in MDS<br>and AML  |
|                                                                               | <i>TP53</i>        | Independently associated<br>with a poor prognosis in MDS<br>and AML  |
|                                                                               | <i>DNMT3A</i>      | Adverse effect on outcome in<br>cytogenetically normal AML           |
|                                                                               | <i>ASXL1</i>       | Independently associated<br>with a poor prognosis in MDS<br>and CMML |
|                                                                               | <i>KIT</i>         | Poor prognosis in CBF AML                                            |
|                                                                               | <i>U2AF1</i>       | Associated with a poor<br>prognosis in MDS                           |
|                                                                               | <i>ZRSR2</i>       | Associated with a poor<br>prognosis in MDS                           |

# Θεραπεία

|             |                        |                                                                                                                                        |
|-------------|------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Therapeutic | <i>FLT3</i>            | Tyrosine kinase inhibitors in phase I/II clinical trials for AML (midostaurin, sorafenib, gilteritinib, crenolanib, quizartinib, etc.) |
|             | <i>IDH1/IDH2</i>       | IDH1 and IDH2 anti-metabolite inhibitors in clinical trials for AML (AG-120, AG-221)                                                   |
|             | <i>JAK2, MPL, CALR</i> | Ruxolitinib                                                                                                                            |
|             | <i>BRAF</i>            | Vemuratinib                                                                                                                            |
|             | <i>KIT</i>             | Tyrosine kinase inhibitors in clinical trials for AML and systemic mastocytosis (midostaurin)                                          |

# παρακολούθηση



|                          |                                                            |                                                                              |
|--------------------------|------------------------------------------------------------|------------------------------------------------------------------------------|
| Minimal Residual Disease | Any driver mutations identified in the diagnostic specimen | Mutation persistence after therapy predicts future relapse <sup>16, 17</sup> |
|--------------------------|------------------------------------------------------------|------------------------------------------------------------------------------|

# Γιατί Next-Gen ;;;

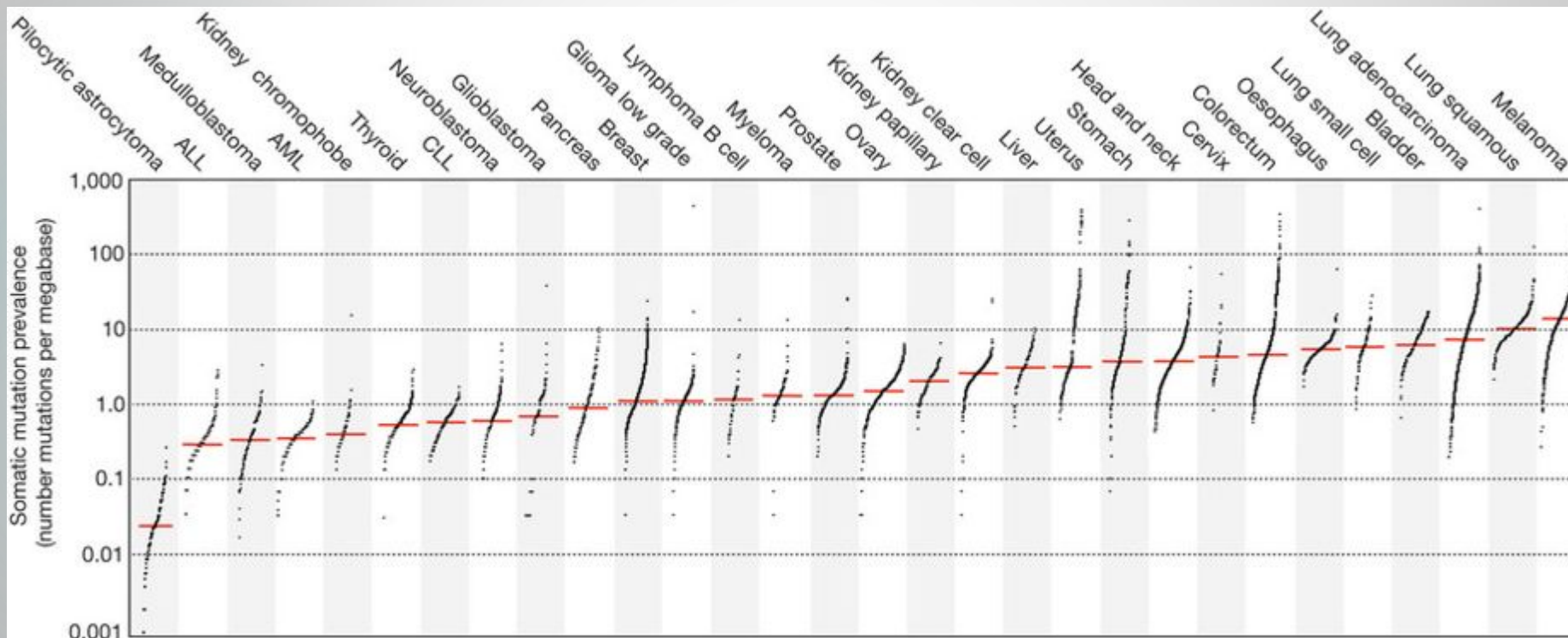
ιατρική ακριβείας  
PRECISION MEDICINE

# Τι είναι η ιατρική ακριβείας ;

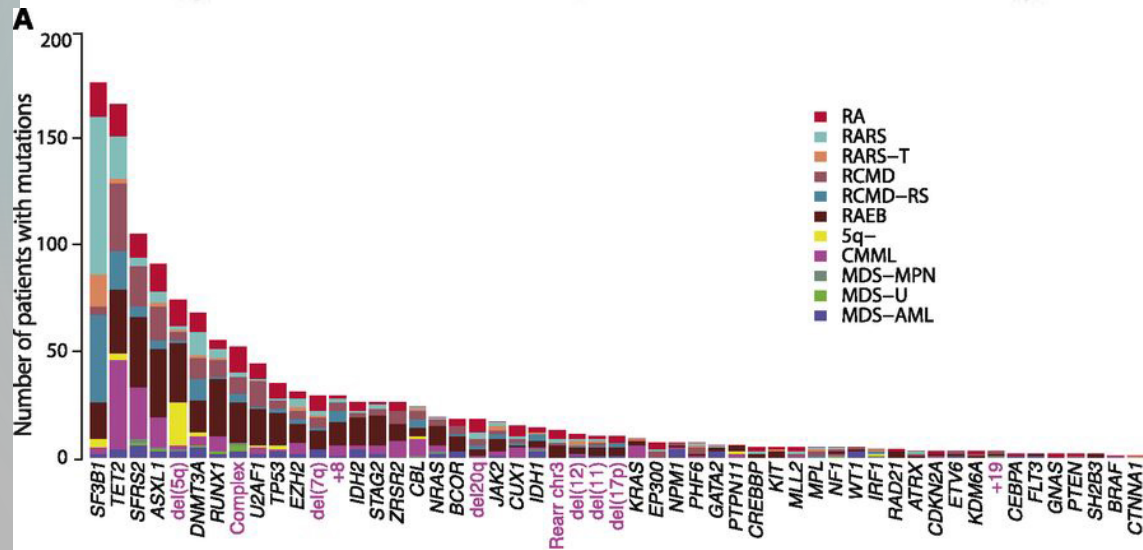
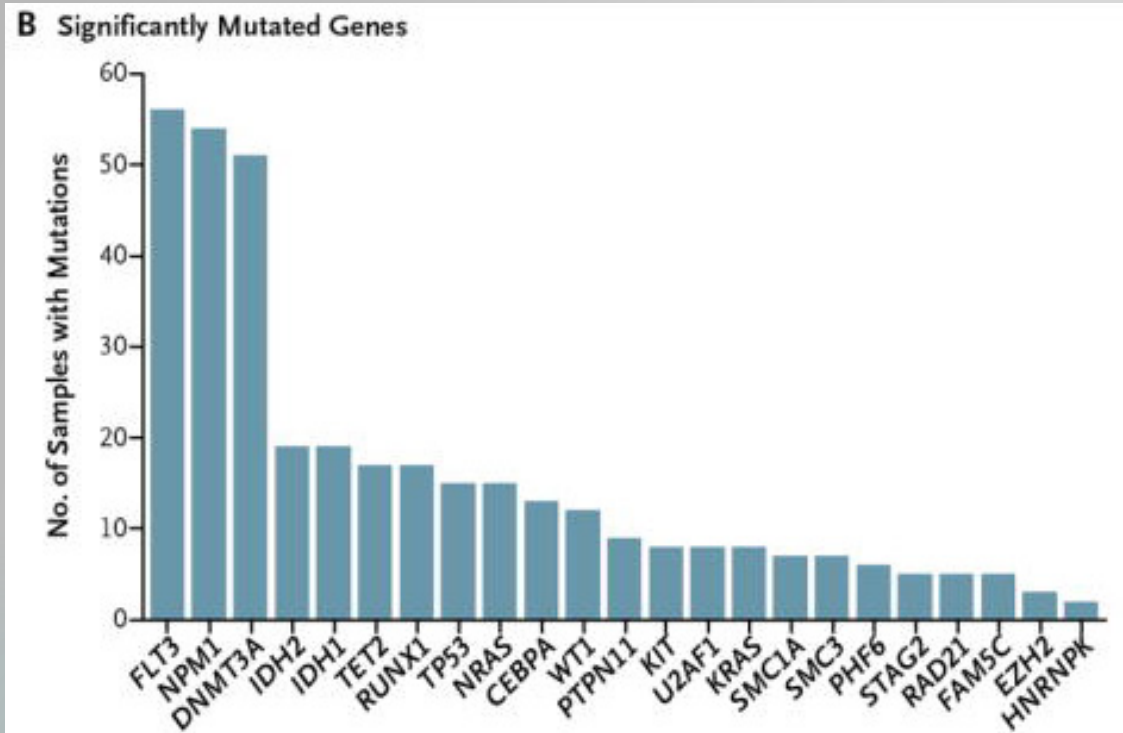
εξατομικευμένη ιατρική περίθαλψη με βάση τα ατομικά χαρακτηριστικά των ασθενών, και την κατάταξή τους σε υποομάδες με βάση παράγοντες όπως η επιθετικότητα της ασθένειας ή η προβλεπόμενη ανταπόκριση στη θεραπεία

# 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

## Βιολογική Εικόνα της Νόσου

23 συχνές «οδηγοί» μεταλλάξεις

237 σπανιότερες

Ανά ασθενή κ.μ.ο 13

## Κατηγορίες μεταλλάξεων

1. Διαμεταθέσεις μεταγραφικών παραγόντων 18%
2. Μεταλλάξεις νουκλεοφωσμίνης 27%
3. Μεταλλάξεις ογκο-κατασταλτικών γονιδίων 16%
4. Μεταλλάξεις γονιδίων που ελέγχουν τη μεθυλίωση του DNA 44%
5. Μεταλλάξεις διεγερτικών γονιδίων σηματοδότησης 59%
6. Τροποποιητές χρωματίνης 30%
7. Αυξητικοί παράγοντες της μυελικής σειράς 22%
8. Μεταλλάξεις γονιδίων στο σύστημα της cohesin 13%
9. Μεταλλάξεις γονιδίων ωρίμανσης RNA (Spliceosome complex) 14%

*The* **NEW ENGLAND**  
**JOURNAL of MEDICINE**

ESTABLISHED IN 1812

MAY 30, 2013

VOL. 363 NO. 23

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 Cancer Journal

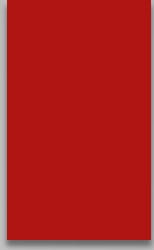
REVIEW ARTICLE

Open Access

# Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use

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

# NGS ροή εργασίας...



## “Wet Bench” διαδικασία

## “Dry Bench” διαδικασία

Προ αναλυτικό στάδιο  
*Pre-analytic*

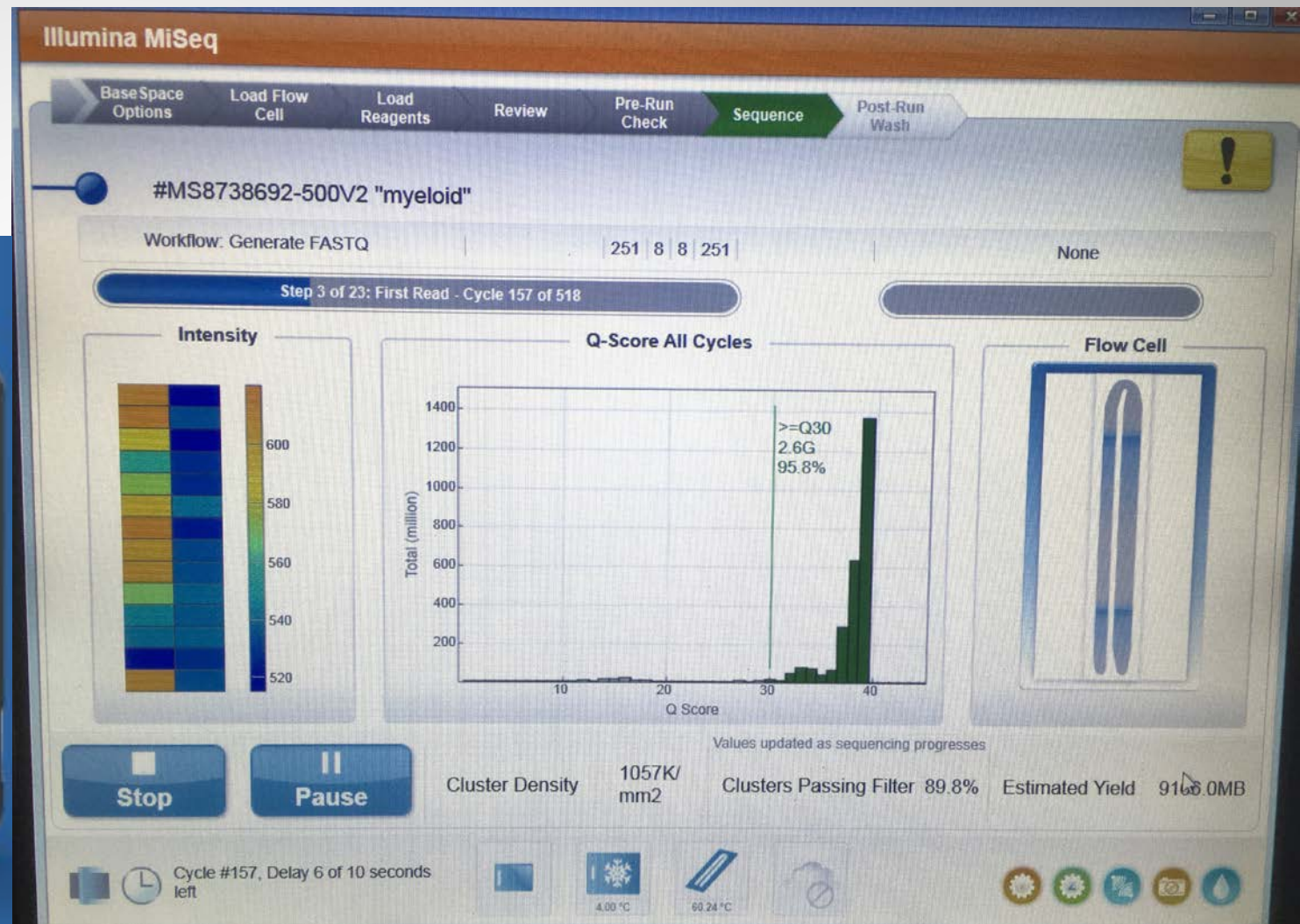
Προετοιμασία  
Βιβλιοθήκης  
*Library Prep*

Προετοιμασία Μήτρας  
*Template Prep*

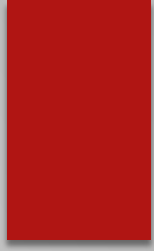
Αλληλούχιση  
*Sequencing*

- Demultiplexing
- Base calling
- Στοιχισή, Alignment
- Variant calling
- Specialized applications
- Variant Annotation (Σχολιασμός)
- Variant significance

# MiSeq



# NGS ροή εργασίας...



## “Wet Bench” διαδικασία

## “Dry Bench” διαδικασία

Προ αναλυτικό στάδιο  
*Pre-analytic*

Προετοιμασία  
Βιβλιοθήκης  
*Library Prep*

Προετοιμασία Μήτρας  
*Template Prep*

Αλληλούχιση  
*Sequencing*

- Demultiplexing
- Base calling
- Στοιχισή, Alignment
- Variant calling
- Specialized applications
- Variant Annotation (Σχολιασμός)
- Variant significance

BaseSpace  
OptionsLoad Flow  
CellLoad  
Reagents

Review

Pre-Run  
Check

Sequence

Post-Run  
Wash

#MS8738692-500V2 "myeloid"

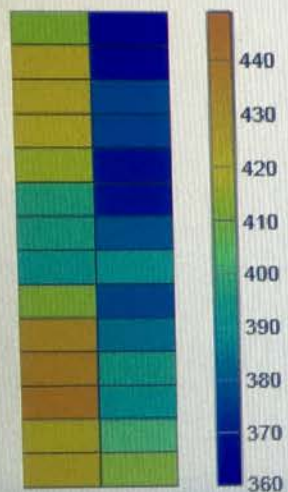
Workflow: Generate FASTQ

251 | 8 | 8 | 251

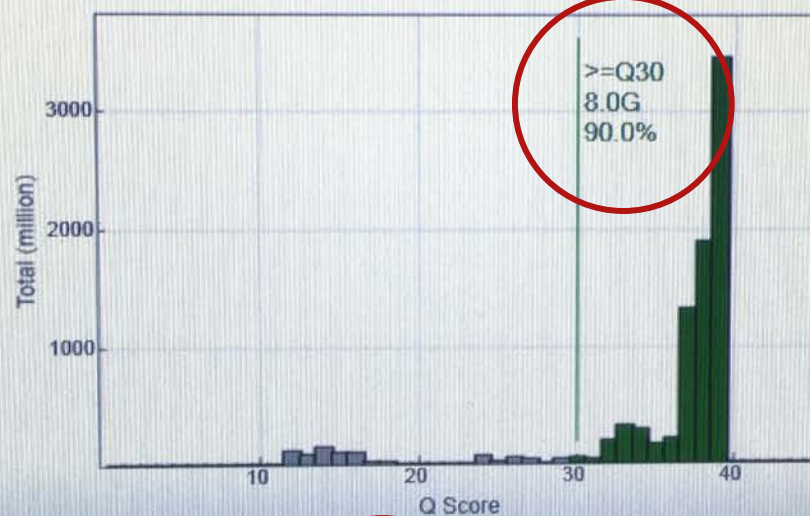
None

Step 20 of 23: Second Read - Cycle 509 of 518

Intensity



Q-Score All Cycles



Flow Cell



Stop

Pause

Cluster Density

1057K/  
mm<sup>2</sup>

Values updated as sequencing progresses

Clusters Passing Filter 89.8%

Estimated Yield 9166.0MB



Cycle #509, Imaging



3.99 °C



19.26 °C



MiSeq

Interactive Biosoftware

ed. Analysis Pending. No analysis.

MYS\_v1: 12

runs: 1 analyses: 12

MYS\_v1: 24

runs: 1 analyses: 24

MYS\_v1: 12

runs: 1 analyses: 12

MYS\_v1: 12

runs: 1 analyses: 12

## Public Databases

Current Versions used in SOPHiA DDM:

|         |            |
|---------|------------|
| ExAC    | r0.3.1     |
| G1000   | 5.20130502 |
| ESP     | 5400       |
| COSMIC  | v83        |
| ClinVar | v20171029  |
| CG69    | 7.20120813 |
| dbSNP   | v150       |
| dbNSF   | v2.9       |

## Pipe

TEST germline  
ROUTINE CE IVD MYS\_v1 somatic

## BDS Numbers

| Lots / Boxes            | Usage       | Expires |
|-------------------------|-------------|---------|
| No BDS number available |             |         |
| No BDS Number           | <div></div> | 2       |

## Support

Operation Manual  
General Information  
Show latest release notes  
Privacy  
Legal

Frequently Asked Question  
Contact Support

## Technical Information

Welcome Maria Garofali

Account Evangelismos  
Account No. 22018  
Client Evangelismos  
Roles [Operator, Ad

DDM https://ddm-  
SGA ch.sophiagene  
SGP ssl.sgp-host.o

Version: 5.3.6--b263-78591f9

## Team

Team Members

Maria Garofalaki  
Brendan Duncan  
Francesca Bomba  
Thomas Petty  
Wenqi You

Internal user  
External user

SOPHiA GENET



01/12/2019

CNV

9 Requests By Date 114 Patients

Filter: 9/9

Status: ☒ All ☒ Completed ☒ In Interpretation ☒ Pipeline Analysis

Ref:

☐ Show cancelled runs

Tests: MYS\_v1, NO\_PIPELINE

[Reset form](#)

[Refresh list](#)

|            |             |            |
|------------|-------------|------------|
| myeloid    | #22018-0009 | 01/12/2019 |
| CNV        | myeloid     | 11/12/2019 |
| myeloid    | #22018-0008 | 16/08/2019 |
| CNV        | myeloid     | 04/12/2019 |
| myeloid    | #22018-0007 | 20/07/2019 |
| CNV        | myeloid     | 07/12/2019 |
| Myeloid    | #22018-0006 | 24/05/2019 |
| CNV        | Myeloid     | 00/24/2019 |
| Myeloid    | #22018-0005 | 22/04/2019 |
| CNV        | Myeloid     | 05/12/2019 |
| Myeloid    | #22018-0004 | 02/03/2019 |
| CNV        | Myeloid     | 05/12/2019 |
| mys        | #22018-0003 | 09/02/2019 |
| CNV        | mys         | 11/12/2019 |
| MYS        | #22018-0002 | 09/11/2018 |
| CNV        | MYS         | 03/12/2018 |
| ???? ????? | #22018-0001 | 27/09/2018 |
| CNV        | ????        | 08/12/2018 |

#22018-0009 myeloid



Sequencer: Illumina MiSeq

Processed date: 01/12/2019

Request date 01/12/2019

26 files



|         |                  |          |                                                  |        |                    |
|---------|------------------|----------|--------------------------------------------------|--------|--------------------|
| #320788 | SAMPLE ID 182523 | MID: S1  | Low coverage! <a href="#">add interpretation</a> |        |                    |
| MYS_v1  | 182523           | Charala  | Interpretation 1 [5]                             | CNVs 1 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320789 | SAMPLE ID 191732 | MID: S2  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191732           | Konstant | Interpretation 1 [4]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320790 | SAMPLE ID 191721 | MID: S3  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191721           | A        | Interpretation 1 [1]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320791 | SAMPLE ID 191980 | MID: S4  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191980           | Mari     | Interpretation 1 [0]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320792 | SAMPLE ID 172020 | MID: S5  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 172020           | Fote     | Interpretation 1 [4]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320793 | SAMPLE ID 180464 | MID: S6  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 180464           | DIAGN    | Interpretation 1 [5]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320794 | SAMPLE ID 191809 | MID: S7  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191809           | RELAP    | Interpretation 1 [5]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320795 | SAMPLE ID 191962 | MID: S8  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191962           | GEORC    | Interpretation 1 [1]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320796 | SAMPLE ID 191804 | MID: S9  | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 191804           | Charal   | Interpretation 1 [2]                             | CNVs 0 | Box: No BDS Number |
| somatic | 01/01/2001       |          |                                                  |        |                    |
| #320797 | SAMPLE ID 192342 | MID: S10 | <a href="#">add interpretation</a>               |        |                    |
| MYS_v1  | 192342           |          | Interpretation 1 [1]                             | CNVs 0 |                    |
| somatic |                  |          |                                                  |        |                    |

SOPHiA DDM

WORKSPACE

Requests

VDB

Variant Database Browser

ANALYSIS 180464

#22018-0009

Evangelismos Hospital-...

Requests

?

+

⚙

⏻

Interpretation 1

SAMPLE #320793 180464 < 6/12 >

RUN 01/12/2019 myeloid

180464 S6 DIAGNOSIS

Overview

OncoPortal

Variants

Patient Pathology (1)

Acute Myeloid Leukemia

REPORTED 5/5

IDH1 JAK2 KIT NPM1 TET2

Myeloid Solution by Sophia

CE IVD

SCREENING GENES

Variant List - sorted by: Prediction>Pathogenicity class>Gene

|     | Actionability | T...  | Gene   | Coding consequence | c.DNA            | Depth | VF%  | ref     | alt     | ClinVar rating        | ref1 | alt1 |
|-----|---------------|-------|--------|--------------------|------------------|-------|------|---------|---------|-----------------------|------|------|
| A 5 |               | INDEL | NPM1   | frameshift         | c.860_863dup     | 6110  | 41.0 | C       | CTCTG   | Pathogenic            | -    | TCTG |
| A 4 |               | SNP   | IDH1   | missense           | c.394C>A         | 8203  | 48.0 | G       | T       | Pathogenic/Likely ... | G    | T    |
| B 3 |               | SNP   | JAK2   | missense           | c.3188G>A        | 6821  | 49.4 | G       | A       | not provided          | G    | A    |
| B 2 |               | SNP   | TET2   | missense           | c.2604T>G        | 8245  | 48.3 | T       | G       | not provided          | T    | G    |
| C 1 |               | SNP   | TET2   | missense           | c.86C>G          | 8440  | 48.8 | C       | G       | not provided          | C    | G    |
| C   |               | SNP   | CEBPA  | synonymous         | c.882C>T         | 8232  | 51.2 | G       | A       |                       | G    | A    |
| C   |               | SNP   | CEBPA  | synonymous         | c.690G>T         | 5582  | 47.0 | C       | A       | Benign                | C    | A    |
| C   |               | SNP   | EZH2   | missense           | c.553G>C         | 6717  | 49.9 | C       | G       | Benign                | C    | G    |
| C   |               | SNP   | EZH2   | intronic           | c.1852-21T>C     | 6975  | 48.6 | A       | G       |                       | A    | G    |
| C   |               | INDEL | EZH2   | 3'UTR              | c.*21del         | 7547  | 99.6 | AGGGGGG | AGGGGGG | Benign                | G    | -    |
| C   |               | SNP   | JAK2   | synonymous         | c.2490G>A        | 5646  | 99.7 | G       | A       | Benign                | G    | A    |
| C   |               | SNP   | JAK2   | synonymous         | c.489C>T         | 4852  | 99.6 | C       | T       | Benign                | C    | T    |
| C   |               | SNP   | JAK2   | intronic           | c.3059+23A>T     | 3590  | 44.8 | A       | T       |                       | A    | T    |
| C   |               | SNP   | KIT    | synonymous         | c.2586G>C        | 8855  | 49.4 | G       | C       | Benign/Likely benign  | G    | C    |
| C   |               | SNP   | RUNX1  | synonymous         | c.1389C>G        | 7231  | 51.9 | G       | C       | Benign                | G    | C    |
| C   |               | SNP   | SETBP1 | synonymous         | c.3825A>G        | 6738  | 49.1 | A       | G       | Benign                | A    | G    |
| C   |               | SNP   | SF3B1  | intronic           | c.2078-8T>A      | 6361  | 48.2 | A       | T       |                       | A    | T    |
| C   |               | INDEL | SF3B1  | intronic           | c.1720-13_172... | 5524  | 99.2 | GAAAA   | GAAAAAA |                       | -    | AA   |
| C   |               | SNP   | SRSF2  | synonymous         | c.144C>T         | 8481  | 99.8 | G       | A       |                       | G    | A    |
| C   |               | SNP   | WT1    | synonymous         | c.471A>G         | 8179  | 49.2 | T       | C       | Benign                | T    | C    |

OVERVIEW

DETAILS

FLAGGING

VIEWER

SIMILAR PATIENTS

WARNINGS

SCREENING

reads 8203 DEPTH 32 min 10984 max

48.0% VARIANT FRACTION

2/12 RUN 4% ACCOUNT .2% COMMUNITY

Pathogenicity

Actionability

0 0 0 0 6

1 2 3 4 5

predictions D C B A

In Report 5

Set To False + 0

transcript NM\_005896.3

Exon rank 4

CDS rank

strand <<<

dbSNP rs121913499

ID ClinVar rs121913499

SNP -21

missense

cDNA c.394C>A

ref/alt G>T

sequence CGT>AGT

amino acid R>S

protein p.(Arg132Ser)

scores

POLYPHEN2 0.878

SIFT 1.0

G1000 N/A

MutationTaster 1.0

ESP5400 N/A

GnomAD N/A

GnomAD

ClinVar

COSMIC

IGV

NCBI

ALAMUT

Google

OMIM

aimlemp@evangelismos-hosp.gr

Evangelismos Hospital-Hematology Dpt

22018

Last login: 13/02/2020 12:41

0, A

SOPHiA CENET CS

5.6.0.3--h2-77580ea






WORKSPACE  
Requests

VDB  
Variant Database Browser

ANALYSIS 180464  
#Z2018-0009

Interpretation 1

SAMPLE #320793 180464 < 6/12 > RUN 01/12/2019 myeloid

Overview

OncoPortal

Variants

SCREENING GENES

SNVs/INDELs

CNVs

FUSIONS

WARNINGS

SOPHIA Filters

Retained Variants 26

Highly Pathogenic A 1

Potentially Pathogenic B 1

Unknown Significance C 18

Likely Benign D 6

Low Confidence Variants 167

Flagged Variants 8

Variant List - sorted by: Prediction>Pathogenicity class>Gene

| P | A | P... | ★ | ! | Actionability | T...   | Gene       | Coding consequence |
|---|---|------|---|---|---------------|--------|------------|--------------------|
| A | 5 | ★    |   |   | INDEL         | NPM1   | frameshift |                    |
| A | 4 | ★    |   |   | SNP           | IDH1   | missense   |                    |
| B | 3 | ★    |   |   | SNP           | JAK2   | missense   |                    |
| B | 2 | ★    |   |   | SNP           | TET2   | missense   |                    |
| C | 1 | ★    |   |   | SNP           | TET2   | missense   |                    |
| C |   |      |   |   | SNP           | CEBPA  | synonymous |                    |
| C |   |      |   |   | SNP           | CEBPA  | synonymous |                    |
| C |   |      |   |   | SNP           | EZH2   | missense   |                    |
| C |   |      |   |   | SNP           | EZH2   | intronic   |                    |
| C |   |      |   |   | INDEL         | EZH2   | 3'UTR      |                    |
| C |   |      |   |   | SNP           | JAK2   | synonymous |                    |
| C |   |      |   |   | SNP           | JAK2   | synonymous |                    |
| C |   |      |   |   | SNP           | JAK2   | intronic   |                    |
| C |   |      |   |   | SNP           | KIT    | synonymous |                    |
| C |   |      |   |   | SNP           | RUNX1  | synonymous |                    |
| C |   |      |   |   | SNP           | SETBP1 | synonymous |                    |
| C |   |      |   |   | SNP           | SF3B1  | intronic   |                    |
| C |   |      |   |   | INDEL         | SF3B1  | intronic   |                    |
| C |   |      |   |   | SNP           | SRSF2  | synonymous |                    |
| C |   |      |   |   | SNP           | WT1    | synonymous |                    |

OVERVIEW

DETAILS

FLAGGING

VIEWER

SIMILAR PATIENTS

WARNINGS

Unique Depth : 18

Unique refNum : 18

Unique altNum : 18

id : 18

Gene : IDH1

Overlap known : rs121913499

Type : SNP

Coding consequence : missense

Reference Genome : GRCh37/hg19

Chromosome : 2

Genome position : 209113113

Depth : 69

VF% : 0

Exon rank : 1

c.DNA : TATTGCCAACAATGACTTACTTIGATCCCCATAAGCATGACGACCTATGATGATAGGTTTACCCATCCACTCACAAGCC

Protein : Q D G Y A H R G I I I P K V W G S V L R

ref : G

alt1 : T

alt2 : T

alt3 : T

alt4 : T

alt5 : T

alt6 : T

alt7 : T

alt8 : T

alt9 : T

alt10 : T

alt11 : T

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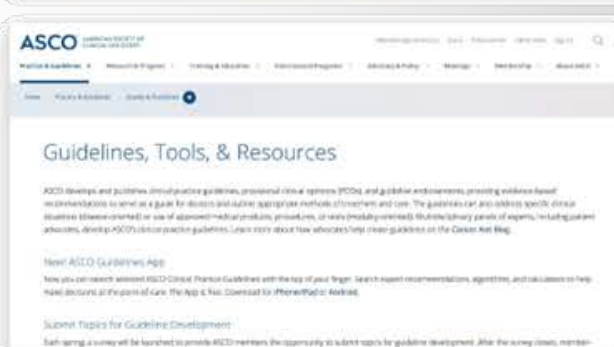
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alt976 : T

- COSMIC
- FDA
- ASCO
- TCGA
- NCCN
- PubMed
- ClinicalTrials.gov
- NCI Drug Dictionary
- LeukemiaNET
- WHO Class of Tumors
- Etc.



# Πολλές πληροφορίες

## information overload





ELSEVIER

Review article

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

Jessica S. Black<sup>a</sup>, Manuel Salto-Tellez<sup>a, b</sup>, Ken I. Mills<sup>a</sup>, Mark A. Catherwood<sup>a, c, \*</sup>

<sup>a</sup> Centre for Cancer Research and Cell Biology (CCRCB), Queen's University Belfast, UK

<sup>b</sup> Tissue Pathology Department, Belfast Health and Social Care Trust, UK

<sup>c</sup> Haematology Department, Belfast Health and Social Care Trust, UK

## ARTICLES

## DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley<sup>1,2,3,4\*</sup>, Elaine R. Mardis<sup>2,3\*</sup>, Li Ding<sup>2,3</sup>, Bob Fulton<sup>3</sup>, Michael D. McLellan<sup>3</sup>, Ken Chen<sup>3</sup>, David Dooling<sup>3</sup>

## 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



## Genomics of AML: Clinical Applications of Next-Generation Sequencing

John S. Welch<sup>1</sup> and Daniel C. Link<sup>1</sup>

<sup>1</sup>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



SPECIAL ARTICLE

Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms

A Report of the Association for Molecular Pathology

Rebecca F. McClure,<sup>†</sup> Mark D. Ewalt,<sup>\*‡</sup> Jennifer Crow,<sup>\*§</sup> Robyn L. Temple-Smolkin,<sup>¶</sup> Mrudula Pullambhatla,<sup>¶</sup> Rachel Sargent,<sup>\*||</sup> and Annette S. Kim<sup>\*\*\*</sup>

Comprehensive genetic diagnosis of acute myeloid leukemia by next-generation sequencing

Elisabeth K.M. Mack,<sup>1</sup> André Marquardt,<sup>1\*</sup> Danny Langer,<sup>1\*</sup> Petra Ross,<sup>1</sup> Alfred Ultsch,<sup>2</sup> Michael G. Kiehl,<sup>3</sup> Hildegard I.D. Mack,<sup>4</sup> Torsten Haferlach,<sup>5</sup> Andreas Neubauer<sup>1</sup> and Cornelia Brendel<sup>1</sup>

<sup>1</sup>Department of Hematology, Oncology and Immunology, Philipps-University Marburg, and University Hospital Gießen and Marburg, Marburg, Germany; <sup>2</sup>Databionics, Department of Mathematics and Informatics, Philipps-University Marburg, Germany; <sup>3</sup>Department of Internal Medicine, Frankfurt (Oder) General Hospital, Frankfurt/Oder, Germany; <sup>4</sup>Institute for Biomedical Aging Research, Leopold-Franzens-University Innsbruck, Austria and <sup>5</sup>MLL, Munich Leukemia Laboratory, Germany

\*These authors contributed equally to this work.



Haematologica 2019  
Volume 104(2):277-287

July 2016 Volume 16, Issue 7, Pages 417–428.

Evaluation of Patients and Families With Concern for Predispositions to Hematologic Malignancies Within the Hereditary Hematologic Malignancy Clinic (HHMC)

Courtney D. DiNardo,<sup>1</sup> Sarah A. Bannon,<sup>2</sup> Mark Routbort,<sup>3</sup> Anna Franklin,<sup>4</sup> Maureen Mork,<sup>2</sup> Mary Armanios,<sup>5</sup> Emily M. Mace,<sup>6</sup> Jordan S. Orange,<sup>6</sup>

PRECISION HEMATOLOGY

Genetic predisposition to hematologic malignancies: management and surveillance

Lucy A. Godley<sup>1,2</sup> and Akiko Shimamura<sup>3</sup>

Blood. 2017;130(4):424-432

N Engl J Med 2018;379:1416-30.

DOI: 10.1056/NEJMoa1716614

ORIGINAL ARTICLE

Classification and Personalized Prognosis in Myeloproliferative Neoplasms

J. Grinfeld, J. Nangalia, E.J. Baxter, D.C. Wedge, N. Angelopoulos, R. Cantrill, A.L. Godfrey, E. Papaemmanuil, G. Gudem, C. MacLean, I. Cook, L. O’Neil.  
Published in final edited form as:

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

Age-related cancer mutations associated with clonal hematopoietic expansion

Mingchao Xie<sup>1,2,\*</sup>, Charles Lu<sup>1,\*</sup>, Jiayin Wang<sup>1,2,\*</sup>, Michael D. McLellan<sup>1</sup>, Kimberly J.



# Initial Diagnostic Workup of Acute Leukemia

NCCN

National  
Comprehensive  
Cancer  
Network®

## Guideline From the College of American Pathologists and the American Society of Hematology

NCCN

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

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

## Acute Myeloid Leukemia, Version 3.2017

Clinical Practice Guidelines in Oncology

Hartmut Döhner,<sup>1</sup> Elihu Estey,<sup>2</sup> David Grimwade,<sup>3</sup> Sergio Amadori,<sup>4</sup> Frederick R. Appelbaum,<sup>2</sup> Thomas D. Schiffer,<sup>5</sup> Hervé Dombret,<sup>6</sup> Benjamin L. Ebert,<sup>7</sup> Pierre Fenaux,<sup>8</sup> Richard A. Larson,<sup>9</sup> Ross L. Levine,<sup>10</sup> Frances A. Lee,<sup>11</sup> Tomoki Naoe,<sup>11</sup> Dietger Niederwieser,<sup>12</sup> Gert J. Ossenkoppele,<sup>13</sup> Miguel Sanz,<sup>14</sup> Jorge Sierra,<sup>15</sup> Marianne B. Kvaloy,<sup>16</sup> Hwei-Fang Tien,<sup>16</sup> Andrew H. Wei,<sup>17,18</sup> Bob Löwenberg,<sup>19</sup> and Clara D. Bloomfield<sup>20</sup>

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;

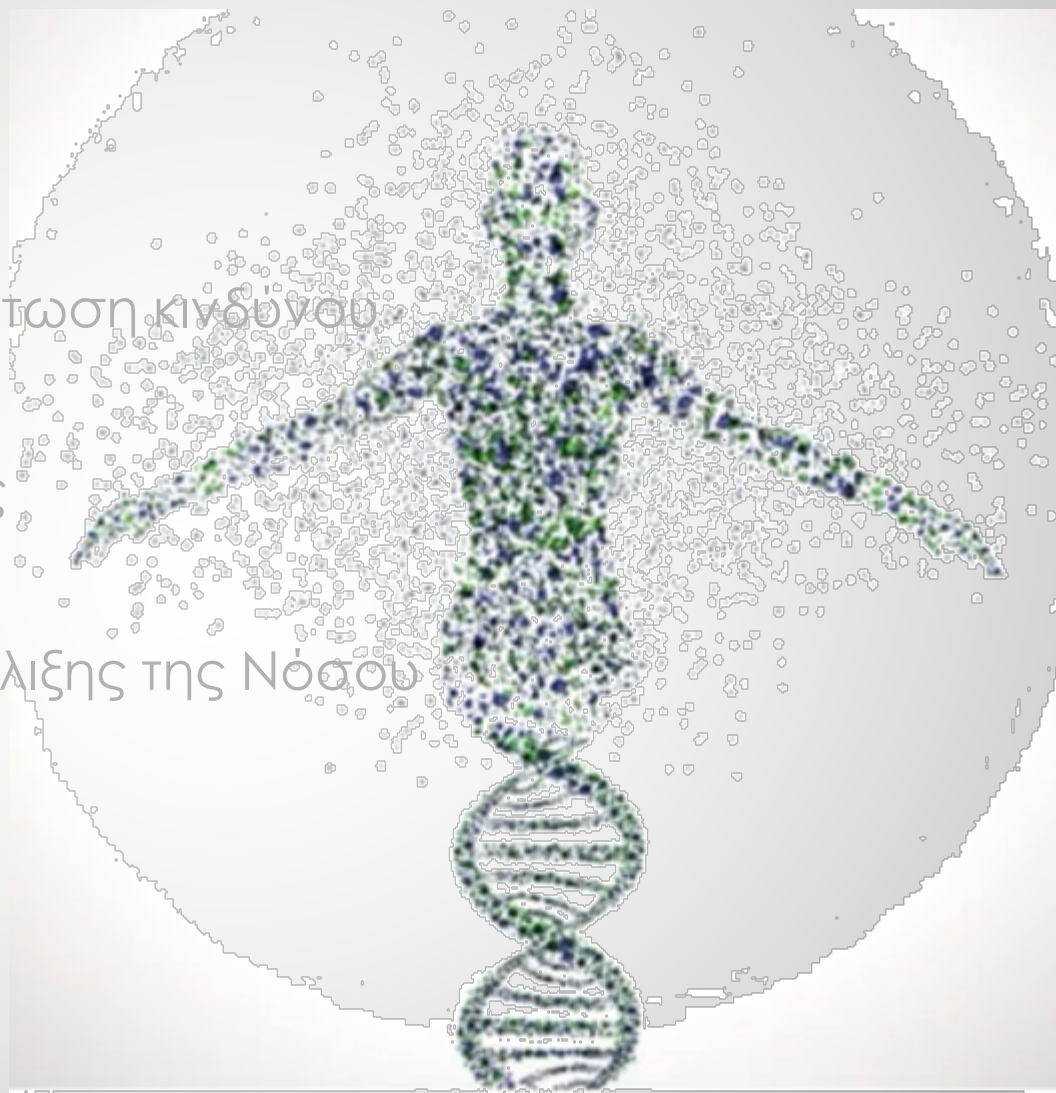
## THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

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

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

# Next Gen στη διάγνωση?

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



# Acute myeloid leukemia (AML)

December 2019

IF Suspicion of AML based on WHO criteria

AM3L

**TEST** (Molecular) cytogenetics/molecular test for detection of large genomic aberrations

AND

**TEST** Gene mutations: ASXL1, CEBPA, DNMT3A, FLT3, IDH1/2, KIT, NPM1, RUNX1, TET2, TP53, WT1

*PML-RARA*

*CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL*

Diagnosis and prognosis

Genetic counseling if a myeloid neoplasm with germline predisposition is suspected

Treatment

*markers: CBFB-MYH11, RUNX1-RUNX1T1, PML-RARA, BCR-ABL, NPM1mut*

Follow-up

If relapse/refractory

**TEST** Gene mutations: ASXL1, CEBPA, DNMT3A, FLT3, IDH1/2, KIT, NPM1, RUNX1, TET2, TP53, WT1

Ασθενής με ΟΜΛ

Διάγνωση: 02/2015

*NPM1*mutA: pos

*FLT3*ITD: pos

Follow up



*NPM1*mutA

Ασθενής με ΟΜΛ

Υποτροπή: 09/2018

*NPM1*mutA: neg

*FLT3*ITD: neg

Ασθενής με ΟΜΛ  
Διάγνωση 02/2015  
*NPM1*mutA: pos  
*FLT3*ITD: pos



WORKSPACE  
Patients

VDB  
Variant Database Browser

ANALYSIS 150218  
#22018-0001

Interpretation 1  
SAMPLE #198679 8 < 8/8 >  
RUN 27/09/2018  
150218 S8 ELENIT

Overview  
OncoPortal  
Variants

SCREENING GENES

Variant List - sorted by: Prediction>Pathogenicity class>Gene

| P | P...         | Actionability | T...  | Gene   | Coding consequence | c.DNA            | Depth | VF%      |
|---|--------------|---------------|-------|--------|--------------------|------------------|-------|----------|
| A | 5            | ★             | INDEL | NPM1   | frameshift         | c.860_863dup...  | 10763 | 41.0 C   |
| A | ★            | ★             | INDEL | FLT3   | inframe_27         | c.1802_1803ins27 | 9013  | 38.2 G   |
| A | ★            | T2 T3 T4 D P  | SNP   | IDH2   | missense           | c.419G>A         | 11047 | 47.0 C   |
| B | 3            | ★             | INDEL | CEBP   | inframe_3          | c.564_566dup...  | 5525  | 1.1 G    |
| B | ★            | ★             | INDEL | CEBPA  | inframe_3          | c.311_313dup...  | 4863  | 1.3 T    |
| C | 1            | ★             | SNP   | SETBP1 | missense           | c.691G>C         | 2326  | 49.4 G   |
| C | 1            | ★             | SNP   | TET2   | missense           | c.5162T>G        | 1491  | 46.9 T   |
| C | ★            | ★             | SNP   | ABL1   | synonymous         | c.1497A>G        | 10081 | 49.7 A   |
| C | ★            | ★             | SNP   | CSF3R  | synonymous         | c.1260T>C        | 6800  | 99.0 A   |
| C | ★            | ★             | SNP   | CSF3R  | synonymous         | c.1254T>C        | 7020  | 47.2 A   |
| C | T2 T3 T4 D P | ★             | SNP   | DNMT3A | synonymous         | c.27C>T          | 7490  | 48.5 G   |
| C | ★            | ★             | SNP   | EZH2   | intronic           | c.118-14T>A      | 2895  | 1.2 A    |
| C | ★            | ★             | INDEL | EZH2   | 3'UTR              | c.*21delC        | 3749  | 54.4 AGG |
| C | ★            | ★             | SNP   | EZH2   | intronic           | c.1852-21T>C     | 6695  | 49.5 A   |
| C | T2 T3 T4 D P | ★             | SNP   | HRA5   | synonymous         | c.81T>C          | 17267 | 49.4 A   |
| C | ★            | ★             | SNP   | SETBP1 | synonymous         | c.3825A>G        | 1081  | 99.1 A   |
| C | ★            | ★             | INDEL | SF3B1  | intronic           | c.1720-13_172... | 2782  | 99.5 GAA |
| C | ★            | ★             | SNP   | SRSF2  | synonymous         | c.144C>T         | 4267  | 48.3 G   |
| C | ★            | ★             | SNP   | ZRSR2  | synonymous         | c.864C>T         | 10120 | 45.9 C   |
| D | 1            | ★             | SNP   | ASXL1  | missense           | c.2444T>C        | 5291  | 99.5 T   |
| D | T2 T3 T4 D P | ★             | SNP   | TP53   | missense           | c.215C>G         | 12107 | 99.4 G   |

SOPHIA Filters

Retained Variants  
21

Highly Pathogenic  
A  
3

Potentially Pathogenic  
B  
1

Unknown Significance  
C  
13

Likely Benign  
D  
4

Low Confidence Variants  
651

Flagged Variants  
8

WORKSPACE  
Patients

VDB  
Variant Database Browser

ANALYSIS 181647  
#22018-0002

Interpretation 1  
SAMPLE #210245 181647 < 6/12 >  
RUN 09/11/2018  
181647 S6

Overview  
OncoPortal  
Variants

SCREENING GENES

Variant List - sorted by: Prediction

| P | P...         | Actionability | T...  | Gene   | Coding consequence | c.DNA            | Depth | VF%            | ref |
|---|--------------|---------------|-------|--------|--------------------|------------------|-------|----------------|-----|
| A | 5            | ★             | SNP   | RUNX1  | nonsense           | c.667G>T         | 3058  | 46.3 C         |     |
| A | ★            | T2 T3 T4 D P  | INDEL | IDH2   | missense           | c.419G>A         | 2964  | 46.1 C         |     |
| A | ★            | ★             | INDEL | ASXL1  | frameshift         | c.1934dupG       | 3281  | 42.0 AGGGGGGGG |     |
| B | 3            | ★             | SNP   | SRSF2  | missense           | c.284C>T         | 3039  | 45.7 G         |     |
| B | 2            | ★             | INDEL | CBL    | inframe_3          | c.1380_1382de... | 3876  | 3.7 TATG       |     |
| B | 3            | ★             | INDEL | TET2   | inframe_3          | c.4624_4626de... | 2255  | 1.1 CCAG       |     |
| C | ★            | ★             | SNP   | SETBP1 | synonymous         | c.3825A>G        | 1930  | 100.0 A        |     |
| C | ★            | ★             | SNP   | CSF3R  | synonymous         | c.1260T>C        | 2634  | 99.9 A         |     |
| C | ★            | ★             | INDEL | SF3B1  | intronic           | c.1720-13_172... | 1168  | 98.8 GAAAA     |     |
| C | ★            | ★             | INDEL | EZH2   | 3'UTR              | c.*21delC        | 2687  | 54.3 AGGGGGG   |     |
| C | 1            | ★             | SNP   | SETBP1 | missense           | c.691G>C         | 3423  | 52.8 G         |     |
| C | T2 T3 T4 D P | ★             | SNP   | DNMT3A | synonymous         | c.27C>T          | 2620  | 49.9 G         |     |
| C | T2 T3 T4 D P | ★             | SNP   | HRA5   | synonymous         | c.81T>C          | 3111  | 49.1 A         |     |
| C | ★            | ★             | SNP   | SRSF2  | synonymous         | c.144C>T         | 3649  | 48.8 G         |     |
| C | ★            | ★             | SNP   | EZH2   | intronic           | c.1852-21T>C     | 2880  | 48.4 A         |     |
| C | ★            | ★             | SNP   | ABL1   | synonymous         | c.1497A>G        | 3904  | 47.8 A         |     |
| C | ★            | ★             | SNP   | ZRSR2  | synonymous         | c.864C>T         | 3422  | 47.0 C         |     |
| C | ★            | ★             | SNP   | CSF3R  | synonymous         | c.1254T>C        | 2699  | 45.1 A         |     |
| C | 1            | ★             | SNP   | TET2   | missense           | c.5162T>G        | 3493  | 44.1 T         |     |
| D | 1            | ★             | SNP   | ASXL1  | missense           | c.2444T>C        | 3402  | 100.0 T        |     |

SOPHIA Filters

Retained Variants  
21

Highly Pathogenic  
A  
3

Potentially Pathogenic  
B  
0

Unknown Significance  
C  
13

Likely Benign  
D  
5

Low Confidence Variants  
103

Flagged Variants  
9

OVERVIEW

DETAILS

FLAGGING

VIEWER

SIMILAR PATIENTS

WARNINGS

SCREENING

reads  
3058  
DEPTH  
45 min 3904 max

frequencies  
1/12  
RUN  
0%  
ACCOUNT  
.0%  
COMMUNITY

Pathogenicity Actionability  
0 0 0 0 0  
1 2 3 4 5  
predictions  
D C B A  
In Report 1  
Set To False + 0

transcript NM\_001754  
cDNA c.667G>T  
ref/alt C>A  
sequence GAG>TAG  
amino acid E>I  
protein p.Glu223\*  
dbSNP -54  
SNP 7-7  
nonsense

strand <<<  
ID ClinVar  
POLYPHEN2 N/A  
SIFT N/A  
G1000 N/A

Ασθενής

ΜΔΣ ή απλαστική

Myeloid panel



RNA Splicing: Epigenetic:

- *SF3B1*
- *SRSF2*
- *U2AF1*
- *ZRSR2*
- *IDH1/2*
- *EZH2*
- *ASXL1*
- *DNMT3A*

SCREENING

GENES

SOPHiA Filters

Retained Variants

27

Highly Pathogenic

0

Potentially Pathogenic

0

Unknown Significance

19

Likely Benign

8

Low Confidence Variants

153

Flagged Variants

7

Variant List - sorted by:

| SOPHiA | Actinability | Pathogenicity | In Report | False+ | Warning | Genomic                    |            | Sample           |                   | VF(%)<br>Depth<br>Filter | Gene<br>Transcript<br>Strand | Transcript             |                |                 |                             | Scores                |                                  | Frequencies                |                               | Cosmic                   | Clinvar                    |                            |
|--------|--------------|---------------|-----------|--------|---------|----------------------------|------------|------------------|-------------------|--------------------------|------------------------------|------------------------|----------------|-----------------|-----------------------------|-----------------------|----------------------------------|----------------------------|-------------------------------|--------------------------|----------------------------|----------------------------|
|        |              |               |           |        |         | Type<br>Chrom.<br>Position | ref<br>alt | reflum<br>altlum |                   |                          |                              | refSeq<br>altSeq       | refAA<br>altAA | CDNA<br>Protein | Exon Rank<br>Consequence    | Exon<br>Position      | Sift<br>Polyphen2<br>Mut. Taster | GnomAD<br>G1000<br>ESP5400 | Community<br>Account<br>(Run) | Link<br>Type             | Link<br>Rating             |                            |
| C      |              |               |           |        |         | SNP                        | chr 4      | 106155199        | C<br>T            | 2156<br>1976             | 48.8%<br>4132<br>->>>        | TET2<br>NM_017628.4    | CTC<br>TTC     | L<br>F          | c.100C>T<br>p.(Leu34Phe)    | exon 3<br>missense    | 100                              | 1.0<br>0.13<br>0.721       | 0.0154<br>0.0064<br>0.0144    | 0.0397<br>0.0463<br>(1)  | COSM5941242<br>coding      | rs11948941<br>not_provided |
| C      |              |               |           |        |         | SNP                        | chr 2      | 25469913         | C<br>T            | 2486<br>2607             | 49.6%<br>5093<br>-<<<        | DNMT3A<br>NM_022552.4  |                |                 | c.1122+7G>A                 | exon 9<br>intronic    | 7                                |                            | 0.7118<br>0.614<br>0.6846     | 0.7284<br>0.9259<br>(9)  | COSM5468672<br>coding      |                            |
| C      |              |               |           |        |         | SNP                        | chr 18     | 42532693         | C<br>A            | 2193<br>2232             | 49.2%<br>4425<br>->>>        | SETBP1<br>NM_015559.2  | CCT<br>ACT     | P<br>T          | c.3388C>A<br>p.(Pro1130Thr) | exon 4<br>missense    | 613                              | 0.897<br>0.003<br>1.0      | 0.1325<br>0.1186<br>0.0953    | 0.1641<br>0.3148<br>(3)  | COSM5019889<br>coding      | rs1064204<br>Benign        |
| C      |              |               |           |        |         | SNP                        | chr 4      | 106197000        | A<br>G            | 1802<br>1898             | 49.8%<br>3700<br>->>>        | TET2<br>NM_001127208.2 | CAT<br>CGT     | H<br>R          | c.5333A>G<br>p.(His1778Arg) | exon 11<br>missense   | 677                              | 0.99<br>0.918<br>1.0       | 0.0435<br>0.0791<br>0.0584    | 0.0944<br>0.1204<br>(2)  | COSM6494955<br>coding      | rs62621450<br>not_provided |
| C      |              |               |           |        |         | SNP                        | chr 9      | 133755528        | A<br>G            | 2290<br>2111             | 48.4%<br>4401<br>->>>        | ABL1<br>NM_005157.5    | GAA<br>GAG     | E<br>E          | c.1497A>G<br>p.(Glu499=)    | exon 9<br>synonymous  | 17                               |                            | 0.0659<br>0.0857<br>0.1075    | 0.0964<br>0.1204<br>(1)  | COSM5021128<br>coding      |                            |
| C      |              |               |           |        |         | SNP                        | chr 20     | 31025163         | A<br>G            | 1928<br>1490             | 43.4%<br>3418<br>-<<<        | ASXL1<br>NM_015338.5   |                |                 | c.*22A>G                    | exon 13<br>3'UTR      | 1960                             |                            | 0.4062<br>0.4<br>0.2997       | 0.5676<br>0.5278<br>(6)  |                            | rs2295764<br>Benign        |
| C      |              |               |           |        |         | SNP                        | chr 20     | 31022444         | G<br>A            | 2460<br>2175             | 46.5%<br>4635<br>->>>        | ASXL1<br>NM_015338.5   | GGG<br>GGA     | G<br>G          | c.1929G>A<br>p.(Gly643=)    | exon 13<br>synonymous | 210                              |                            | 0.0002<br>-<br>0.0001         | 0.0003<br>0.0093<br>(1)  |                            |                            |
| C      |              |               |           |        |         | SNP                        | chr 20     | 31024274         | T<br>C            | 2518<br>2244             | 47.6%<br>4762<br>-<<<        | ASXL1<br>NM_015338.5   | AGT<br>AGC     | S<br>S          | c.3759T>C<br>p.(Ser1253=)   | exon 13<br>synonymous | 868                              |                            | 0.4182<br>0.4058<br>0.3148    | 0.5977<br>0.5556<br>(6)  | COSM4134435<br>coding      | rs4911231<br>Benign        |
| C      |              |               |           |        |         | SNP                        | chr 1      | 36937059         | A<br>G            | 5<br>3530                | 99.7%<br>3535<br>-<<<        | CSF3R<br>NM_000760.3   | ACT<br>ACC     | T<br>T          | c.1260T>C<br>p.(Thr420=)    | exon 10<br>synonymous | 26                               | 0.0                        | 0.5585<br>0.5925<br>0.6074    | 0.5157<br>0.7222<br>(8)  | COSM1748390<br>coding      | rs3917981<br>Benign        |
| C      |              |               |           |        |         | SNP                        | chr 1      | 36937065         | A<br>G            | 1859<br>1802             | 48.4%<br>3661<br>-<<<        | CSF3R<br>NM_000760.3   | CGT<br>CGC     | R<br>R          | c.1254T>C<br>p.(Arg418=)    | exon 10<br>synonymous | 32                               | 0.94                       | 0.3103<br>0.2464<br>0.2922    | 0.3517<br>0.4815<br>(4)  | COSM4143873<br>coding      | rs3917980<br>Benign        |
| C      |              |               |           |        |         | SNP                        | chr 2      | 25536827         | G<br>A            | 2181<br>1907             | 47.5%<br>4088<br>-<<<        | DNMT3A<br>NM_022552.4  | CCC<br>CCT     | P<br>P          | c.27C>T<br>p.(Pro9=)        | exon 2<br>synonymous  | 27                               |                            | 0.0937<br>0.0948<br>0.0737    | 0.1746<br>0.2315<br>(6)  | COSM3766569<br>coding      |                            |
| C      |              |               |           |        |         | SNP                        | chr 7      | 148508833        | A<br>G            | 5<br>3229                | 99.9%<br>3234<br>-<<<        | EZH2<br>NM_004456.4    |                |                 | c.1852-21T>C                | exon 16<br>intronic   | 21                               |                            | 0.6672<br>0.6907<br>0.6822    | 0.7329<br>0.8704<br>(11) | COSN15654178<br>non coding |                            |
| C      |              |               |           |        |         | INDEL                      | chr 7      | 148504716        | AGGG...<br>AGGGGG | 6<br>3111                | 99.8%<br>3117<br>-<<<        | EZH2<br>NM_004456.4    |                |                 | c.*21del                    | exon 20<br>3'UTR      | 82                               |                            | 0.6901<br>0.771<br>-          | 0.6446<br>0.8889<br>(10) | COSN20104935<br>non coding | rs3217095<br>Benign        |

Ασθενής

ΜΔΣ ή απλαστική

Myeloid panel



απλαστική

RNA Splicing: Epigenetic:

- *SF3B1*
- *SRSF2*
- *U2AF1*
- *ZRSR2*
- *IDH1/2*
- *EZH2*
- *ASXL1*
- *DNMT3A*

# NGS και Μυέλωμα

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

Notes:

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

# CHIP-ICUS-CCUS-Cancer Spectrum

|      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CHIP | <ul style="list-style-type: none"><li>• Ασθενείς χωρίς προηγούμενη ή ταυτόχρονη αιματολογική κακοήθεια</li><li>• Σωματικές μεταλλάξεις σε ένα ή μερικές φορές &gt;1 γονίδιο σε ποσοστό &gt;2% variant allele fraction (VAF)<ul style="list-style-type: none"><li>• <i>TET2, DNMT3A, ASXL1, SF3B1, TP53</i></li></ul></li><li>• Αύξηση επίπτωσης με την ηλικία</li><li>• Αναγνωρίστηκαν με WES/WGS σε μεγάλους πληθυσμούς</li><li>• Κίνδυνος εξέλιξης σε μυελογενή κακοήθεια (0.5-1% /έτος; 10X κινδύνου)</li></ul> |
| ICUS | <ul style="list-style-type: none"><li>• Ανεξήγητη κυτταροπενία με απουσία κυτταρογενετικού και ανιχνεύσιμου με NGS κλωνικού πληθυσμού</li><li>• Αβέβαιος κίνδυνος νεοπλασματικής εξέλιξης, πιθανά χαμηλός</li></ul>                                                                                                                                                                                                                                                                                                |
| CCUS | <p>CHIP + ICUS</p> <ul style="list-style-type: none"><li>• Ο κίνδυνος εξέλιξης σε μυελογενή νεοπλασία είναι υψηλότερος</li></ul>                                                                                                                                                                                                                                                                                                                                                                                   |

# Ενδείξεις για εφαρμογή NGS

## Χρήσιμο

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

## Δεν ενδείκνυται/Αβέβαιο

- ▶ Palliative situation
- ▶ Μαστοκυττάρωση (*KITD816V*, *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 νεοπλασιών
- ▶ στην **πρόγνωση**,
  - ▶ στη **θεραπευτική** (therapeutic-diagnostics)
- ▶ κατανόηση: co-mutation patterns + clonal hematopoiesis

ευχαριστώ

