AKADEMISKA SJUKHUSET

UPPSALA UNIVERSITET

Next generation karyotyping in AML

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Applying whole genome and/or transcriptome sequencing for routine AML diagnostic and prognostication

- Conventional karyotyping is used for routine diagnostics of AML since decades
- Conventional karyotyping has low sensitivity, is time consuming and labor intensive, needs very skilled professionals, a subjective element
- New sequencing techniques provide more sensitive techniques with a potential to address the weaknesses of conventional cytogenetic analysis and give large amount of additional information

REGULAR ARTICLE

blood advances

Challenging conventional karyotyping by next-generation karyotyping in 281 intensively treated patients with AML

Sylvain Mareschal,[1,2,*] Anna Palau,[1,*] Johan Lindberg,[3] Philippe Ruminy,[4] Christer Nilsson,[5,6] Sofia Bengtzén,[5,6] Marie Engvall,[7] Anna Eriksson,[2] Anne Neddermeyer,[2] Vinciane Marchand,[4] Monika Jansson,[5,6] My Björklund,[2] Fabrice Jardin,[4,8] Mattias Rantalainen,[9] Andreas Lennartsson,[1] Lucia Cavelier,[7] Henrik Grönberg,[9] and Sören Lehmann[2,5,6]

Sylvain Mareschal, bioinformatistician, post-doc

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The NEW ENGLAND JOURNAL of MEDICINE

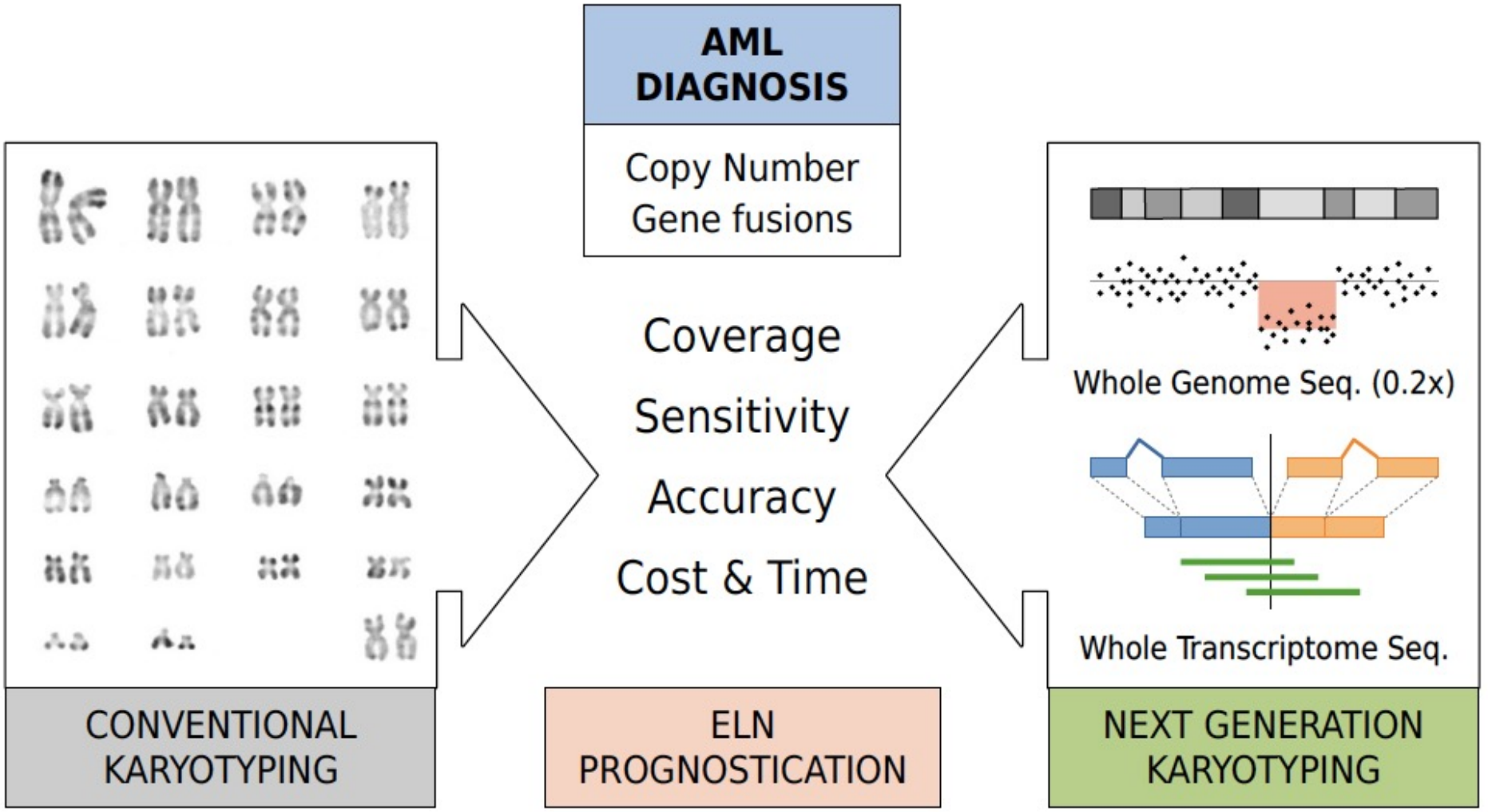
ORIGINAL ARTICLE

Genome Sequencing as an Alternative to Cytogenetic Analysis in Myeloid Cancers

Eric J. Duncavage, M.D., Molly C. Schroeder, Ph.D., Michele O'Laughlin, B.S., Roxanne Wilson, B.S., Sandra MacMillan, B.S., Andrew Bohannon, B.S., Scott Kruchowski, B.S., John Garza, B.S., Feiyu Du, M.S., Andrew E.O. Hughes, M.D., Ph.D., Josh Robinson, B.A., Emma Hughes, B.S., Sharon E. Heath, Jack D. Baty, B.A., Julie Neidich, M.D., Matthew J. Christopher, M.D., Ph.D., Meagan A. Jacoby, M.D., Ph.D., Geoffrey L. Uy, M.D., Robert S. Fulton, M.S., Christopher A. Miller, Ph.D., Jacqueline E. Payton, M.D., Ph.D., Daniel C. Link, M.D., Matthew J. Walter, M.D., Peter Westervelt, M.D., Ph.D., John F. DiPersio, M.D., Ph.D., Timothy J. Ley, M.D., and David H. Spencer, M.D., Ph.D.

Pan cancer sequencing strategy at Karolinska 2014

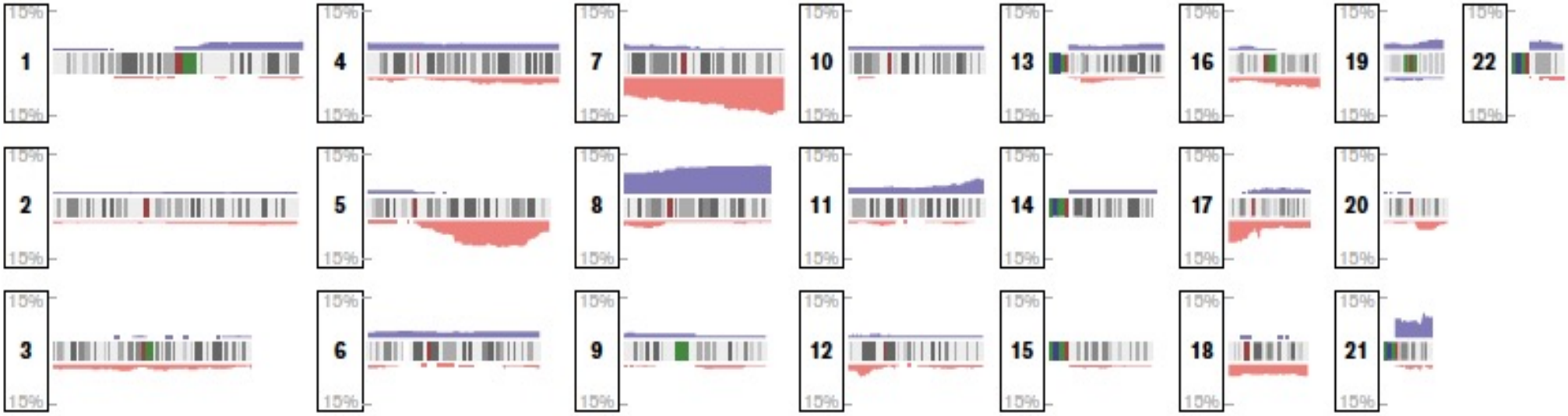
- 400 AML cases
- Deep exome sequencing of 550 cancer and pharmacokinetic genes
- Whole transcriptome sequencing (RNA-seq, 105 bp, pair end, 30X)
- Low pass (shallow) whole genome sequencing (sWGS) (0.2X)


AML DIAGNOSIS
Copy Number
Gene fusions
Coverage
Sensitivity
Accuracy
Cost & Time
Whole Genome Seq. (0.2x)
Whole Transcriptome Seq.
CONVENTIONAL KARYOTYPING
ELN PROGNOSTICATION
NEXT GENERATION KARYOTYPING

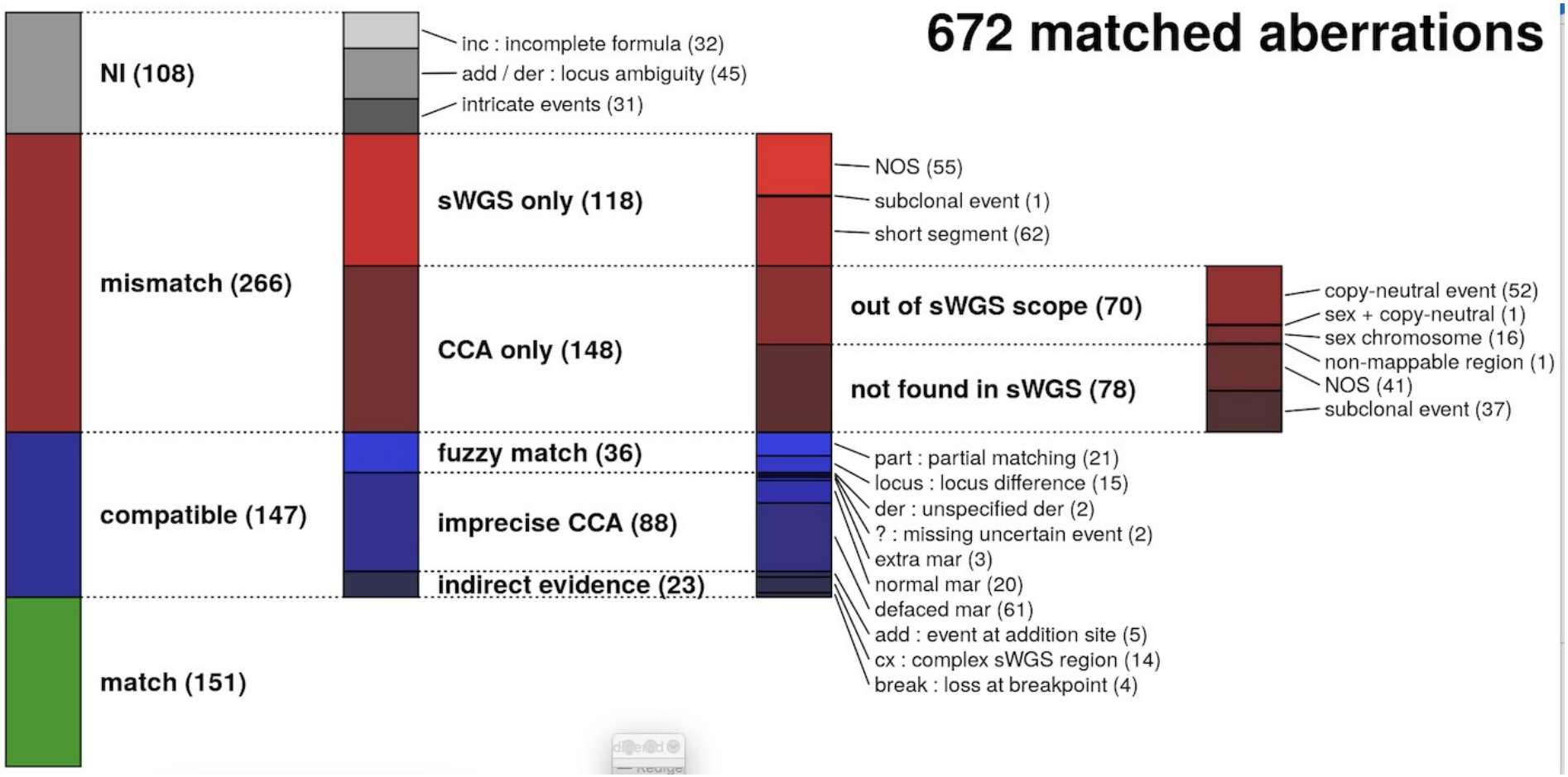
Selection for study of Next Generation Karyotyping (NGK)

- 291 consequetive population-based
  - Median age 66 years (18 to 86)
  - 49.5 % women
- Successful conventional cytogenetic analysis (CCA)
- Validation with FISH for copy number alterantions (CNAs) and LD-RTPCR targeted DNA based technique detecting > 100 known fusions
- Additional data on mutations from targeted exome sequencing

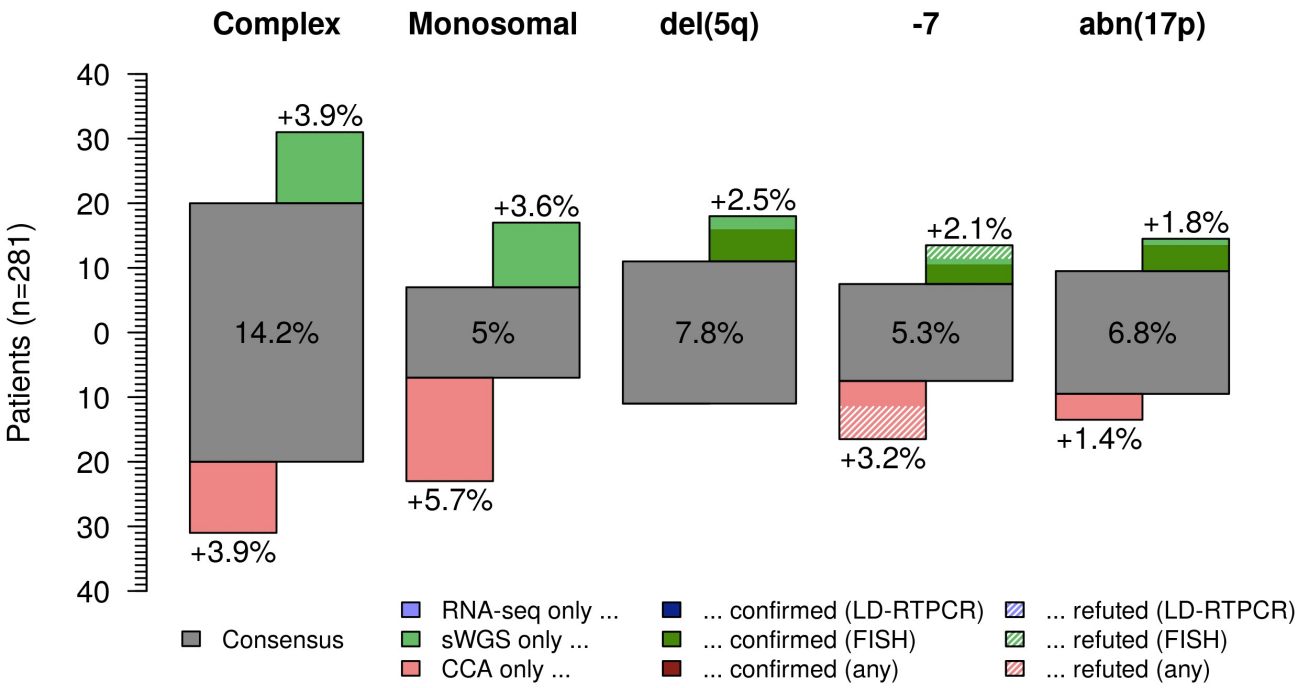
Copy number changes from sWGS



Matching copy number alterantions (CNAs) between sWGS and CCA



Matching ELN defining CNAs between sWGS and CCA



- Concordance between CCA och sWGS 94.3% (Fisher p-values < 1e-9)
- More monosomal karyotypes and monosomy 7 i CCA
- More 5q in sWGS

FISH validation of mismatches of ELN defining mismatched aberrations

ELN conclusion			
criteria	sWGS	CCA	FISH
del(5q)	+	–	+
abn(17p)	+	–	+
del(5q)	+	–	+
abn(17p)	+	–	+
-7	–	+	–
del(5q)	+	–	+
abn(17p)	+	–	+
-7	–	+	–
-7	–	+	–
-7	+	–	+
-7	+	–	+
del(5q)	+	–	+
del(5q)	+	–	+
-7	+	–	+
-7	+	–	–
abn(17p)	+	–	+/–
-7	+	–	–
-7	–	+	–
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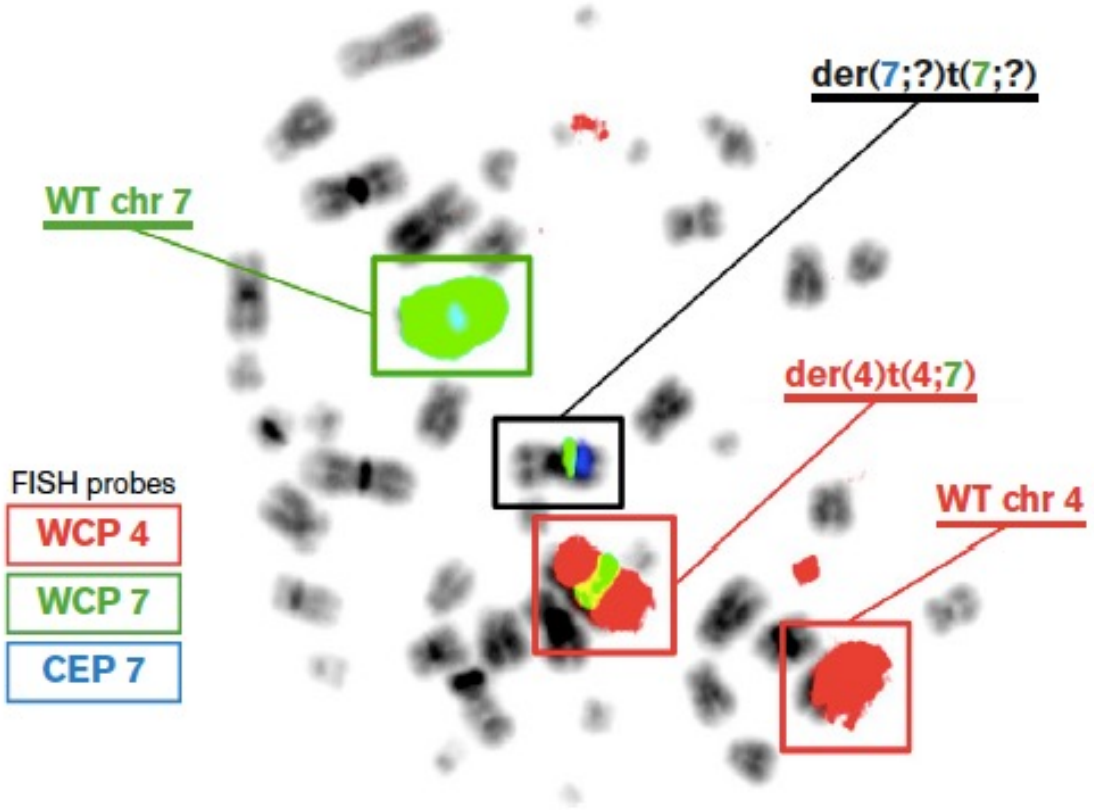
-7	+	–	+
del(5q)	+	–	+
del(5q)	+	–	+
-7	+	–	+
-7	+	–	–
abn(17p)	+	–	+/–
-7	+	–	–
-7	–	+	–
-7	–	+	–
-7	–	+	–



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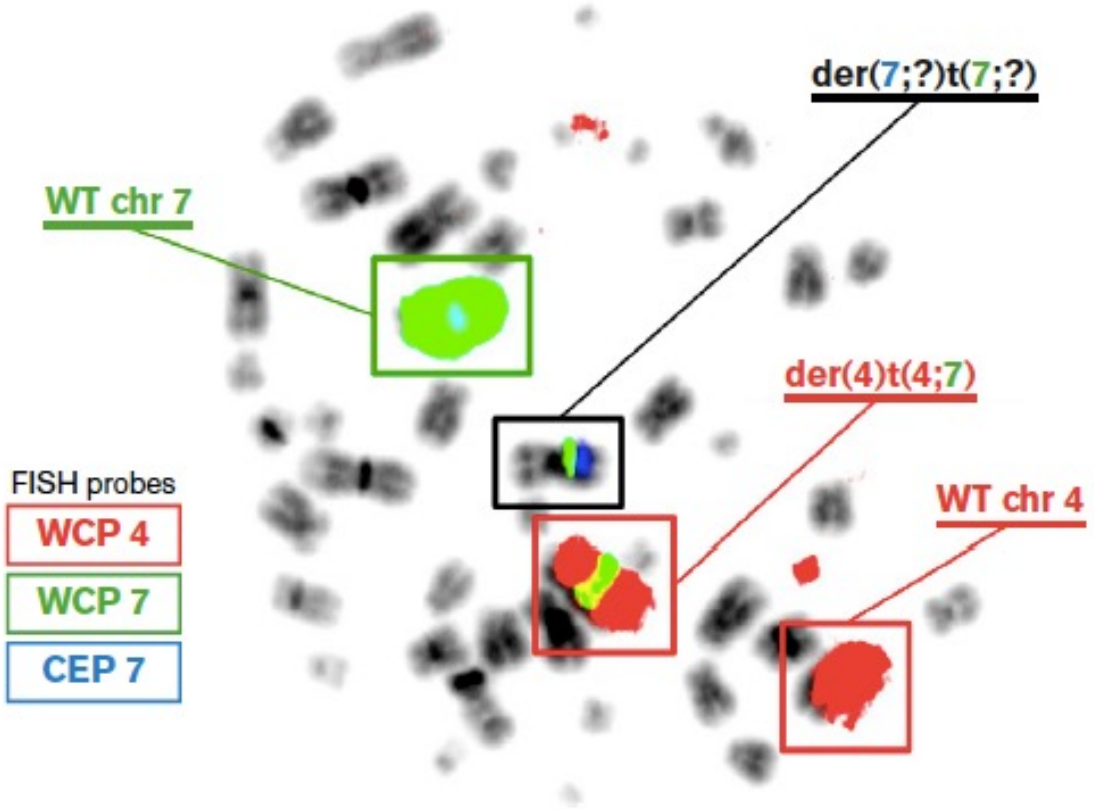
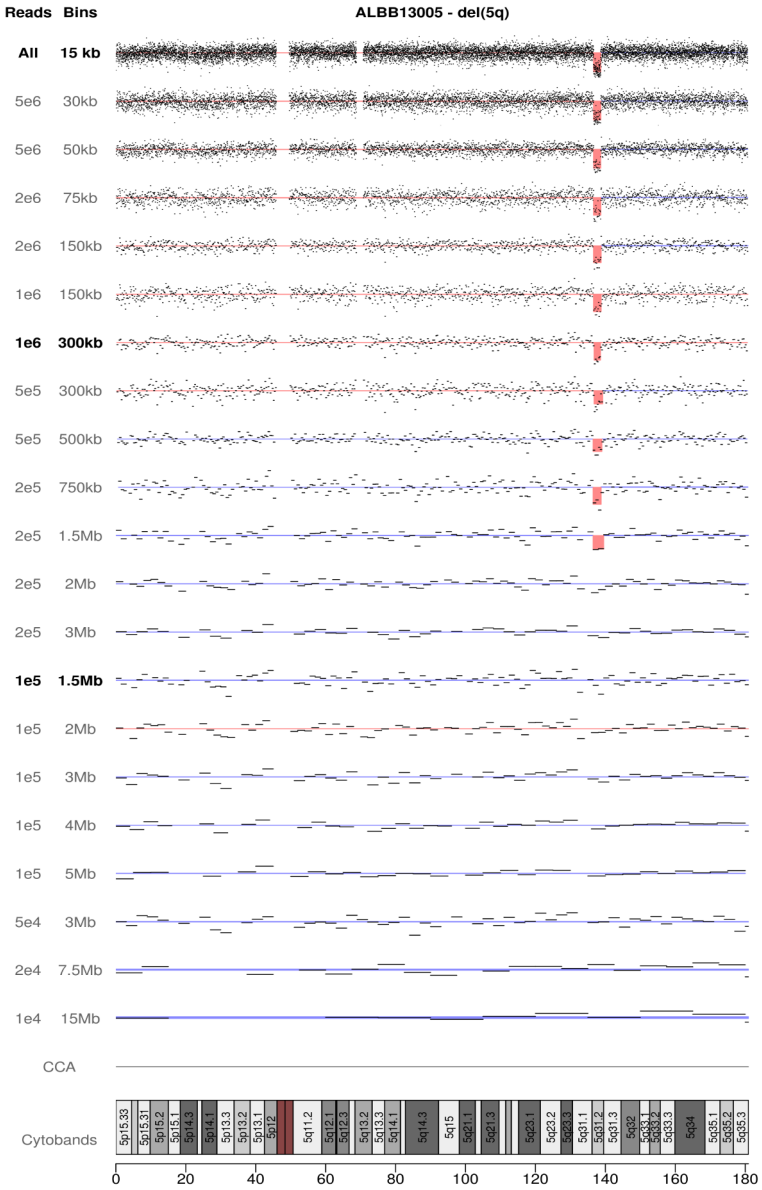
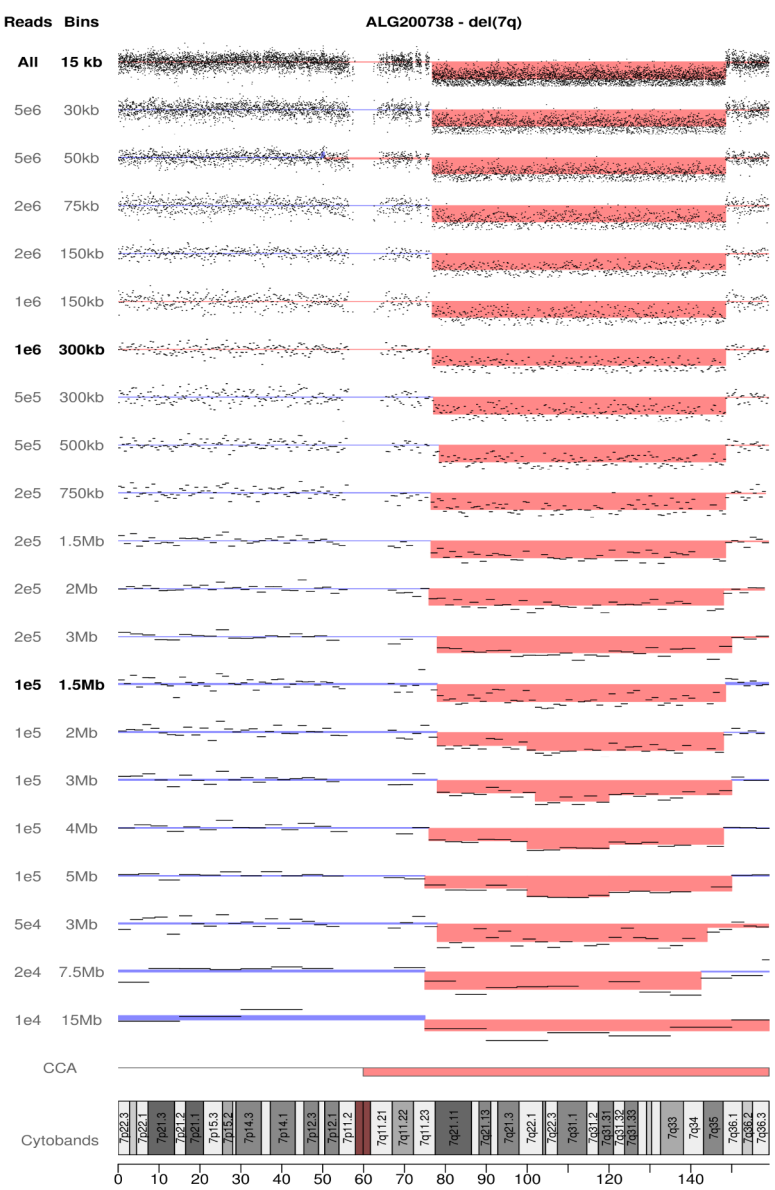


- 61 cases of potential pseudomonosomy were identified
- 67% of monosomy 7 together with at least 1 marker chromosome, or in 33% of all monosomy 7 cases, the monosomy was a pseudomonosomy

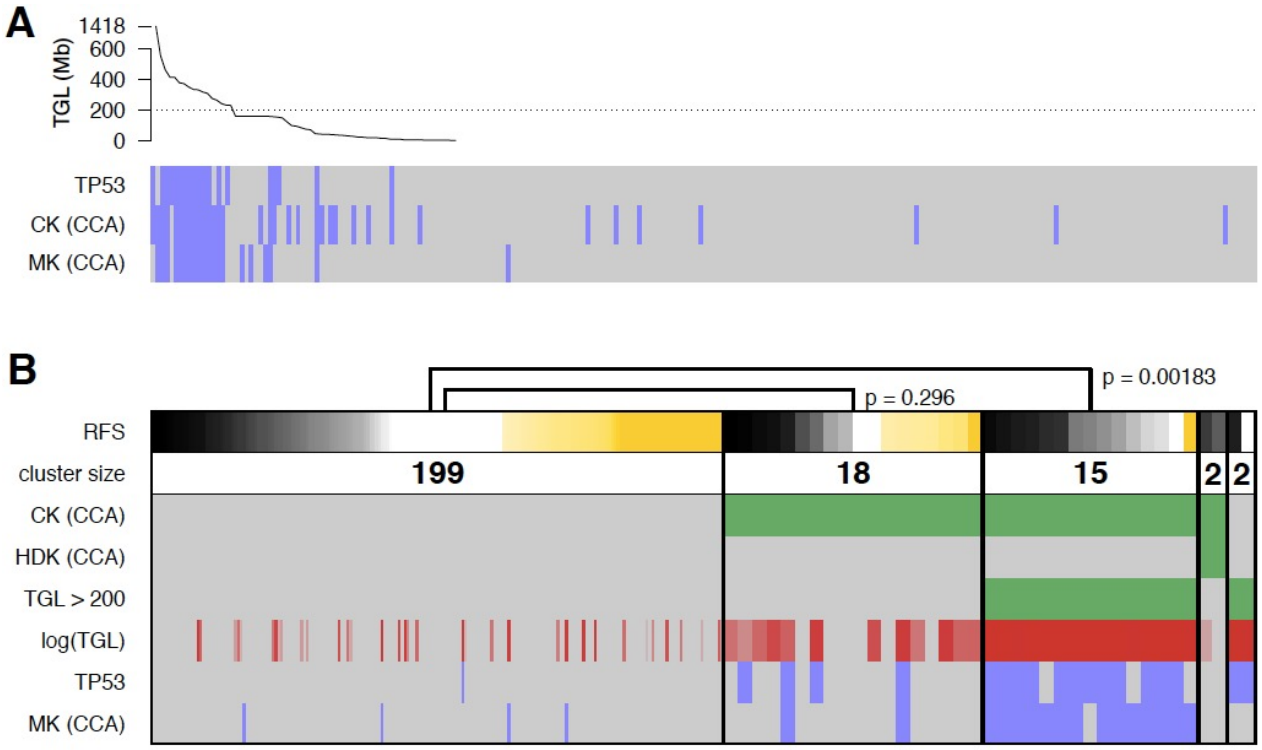




In silico dilutions of sWGS

- Dilutions down to 6 million reads (with 10 times less reads) gives precision level of 98.5%
- For ELN CNAs alone, 6 million reads gives precision level of 100%

sWGS based definition matching complex karyotype



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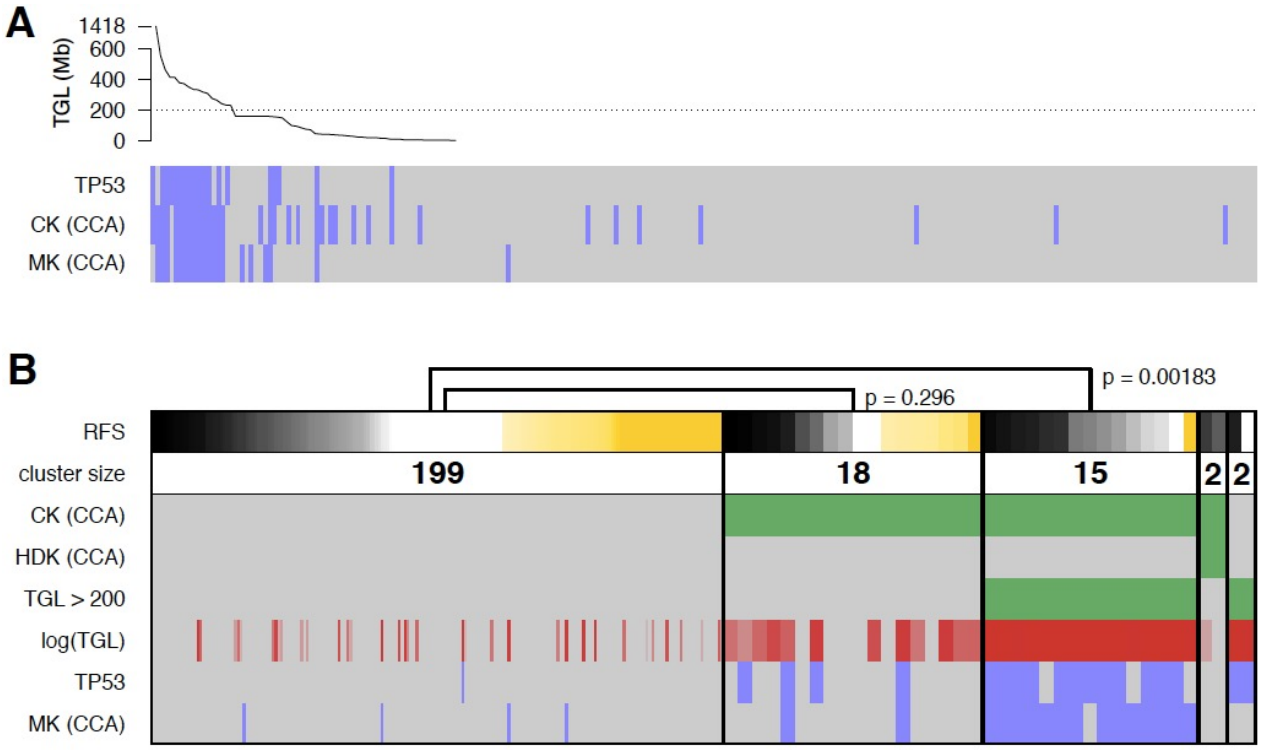
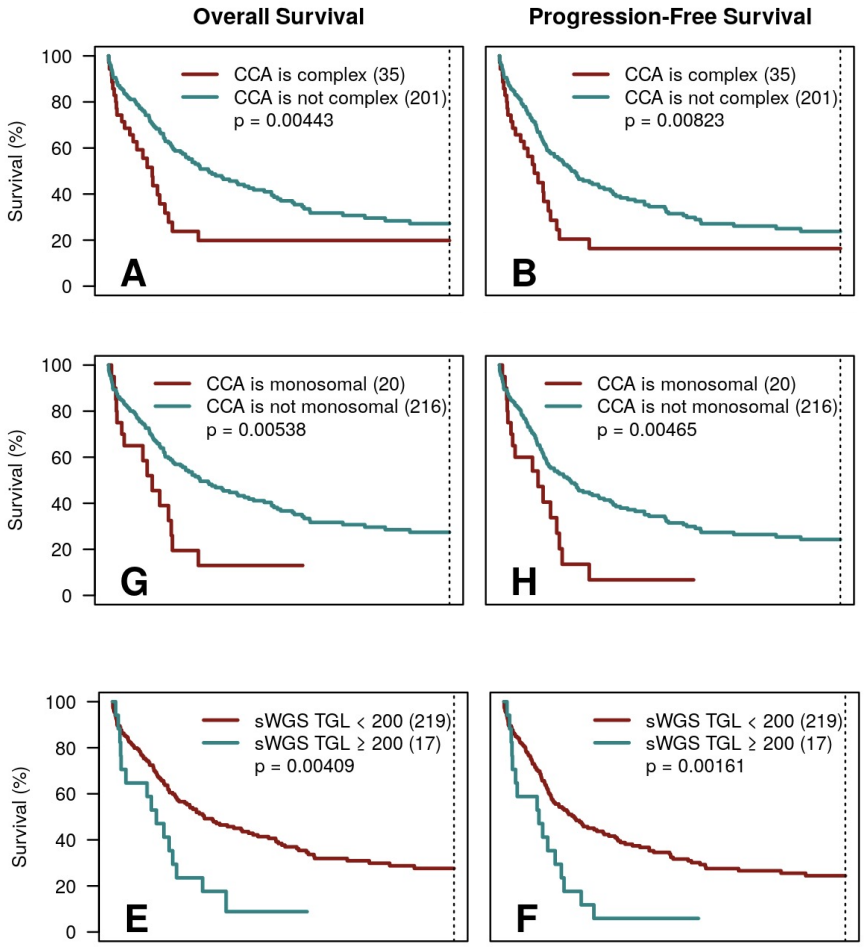
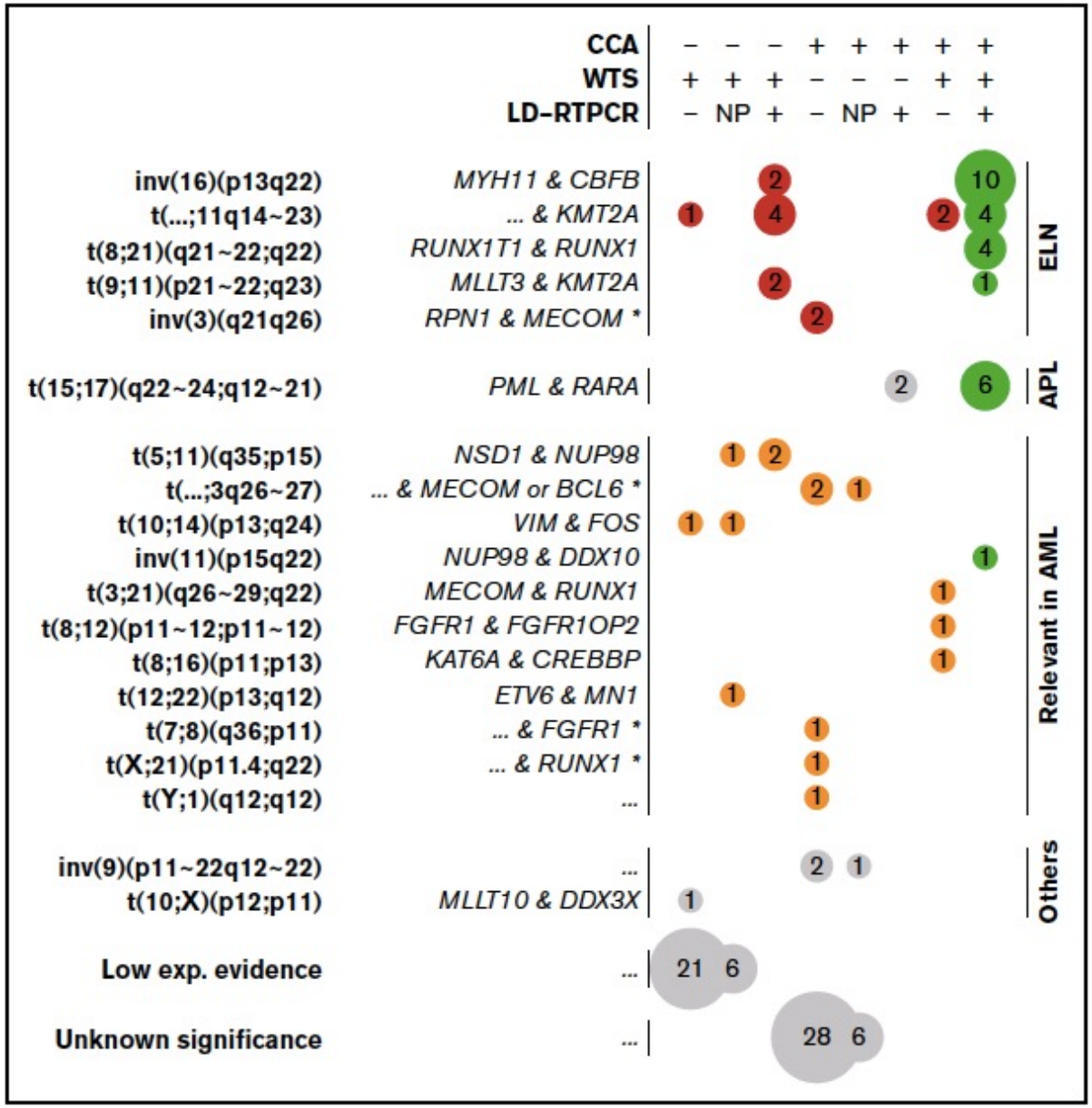
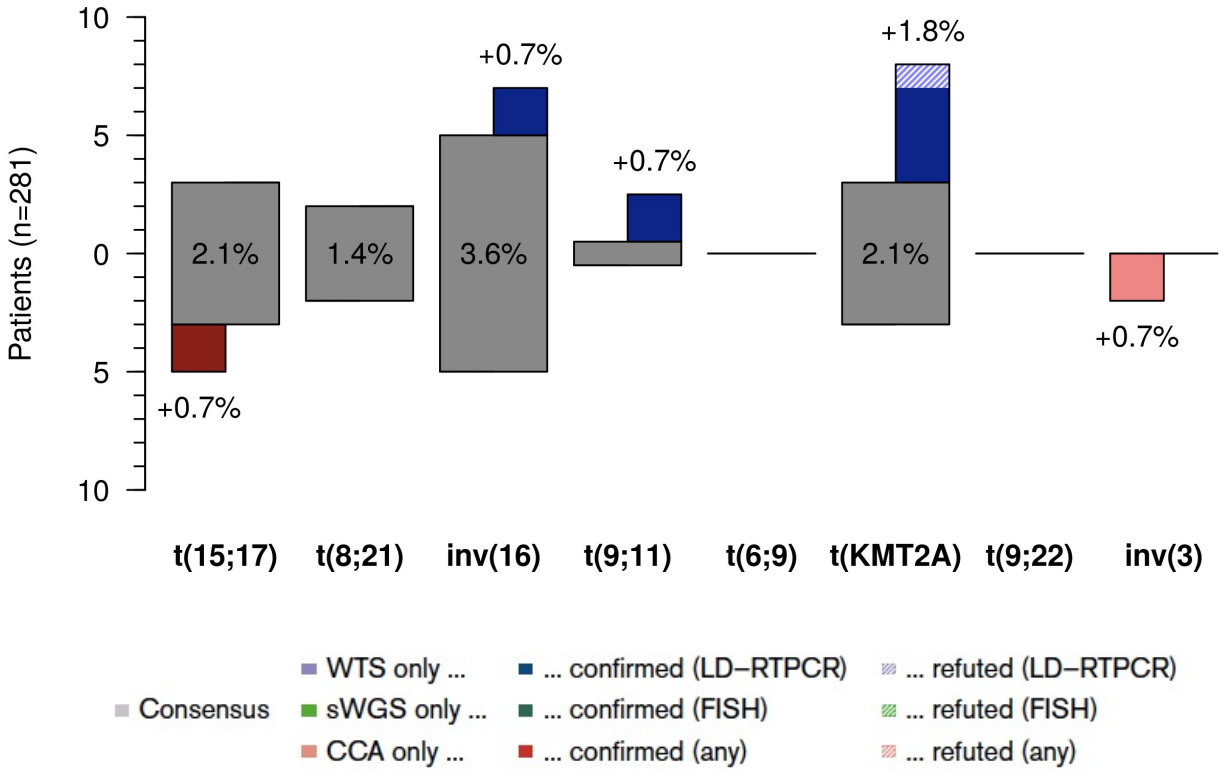






Matching gene fusions using whole transcriptome sequencing

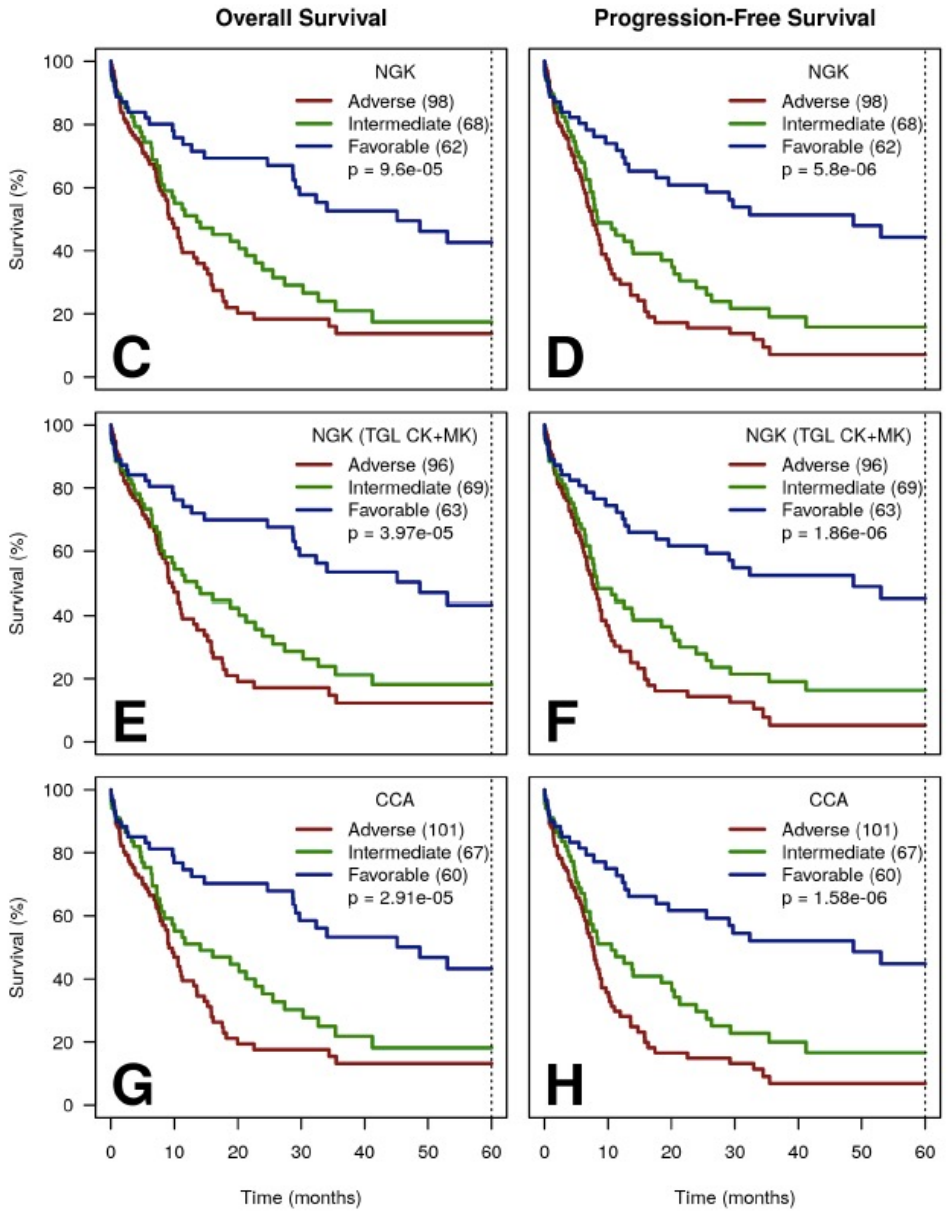
Matching ELN defining fusions



- Good concordance between CCA och WTS for ELN-fusions (99.4%)
- WTS showed better sensitivity for inv(16) (N=2) and *KMT2A* rearrangement (n=7)
- inv(3) missed by WTS due to a fusion without fusion transcript - enhancer switch translocation
- 2 of 8 PML-RARA fusions missed by WTS
  - transcripts present but below thereshold
  - lowly expressed – low blast percentage

Similar results on OS and RFS according to ELN2017 CCA vs NGK

- 17 of 281 (6%) changed risk group with NGK compared to conventional
  - 7 adverse to intermediate
  - 6 intermediate to adverse
  - 2 favorable to intermediate
  - 1 adverse to favorable
  - 1 intermediate to favorable



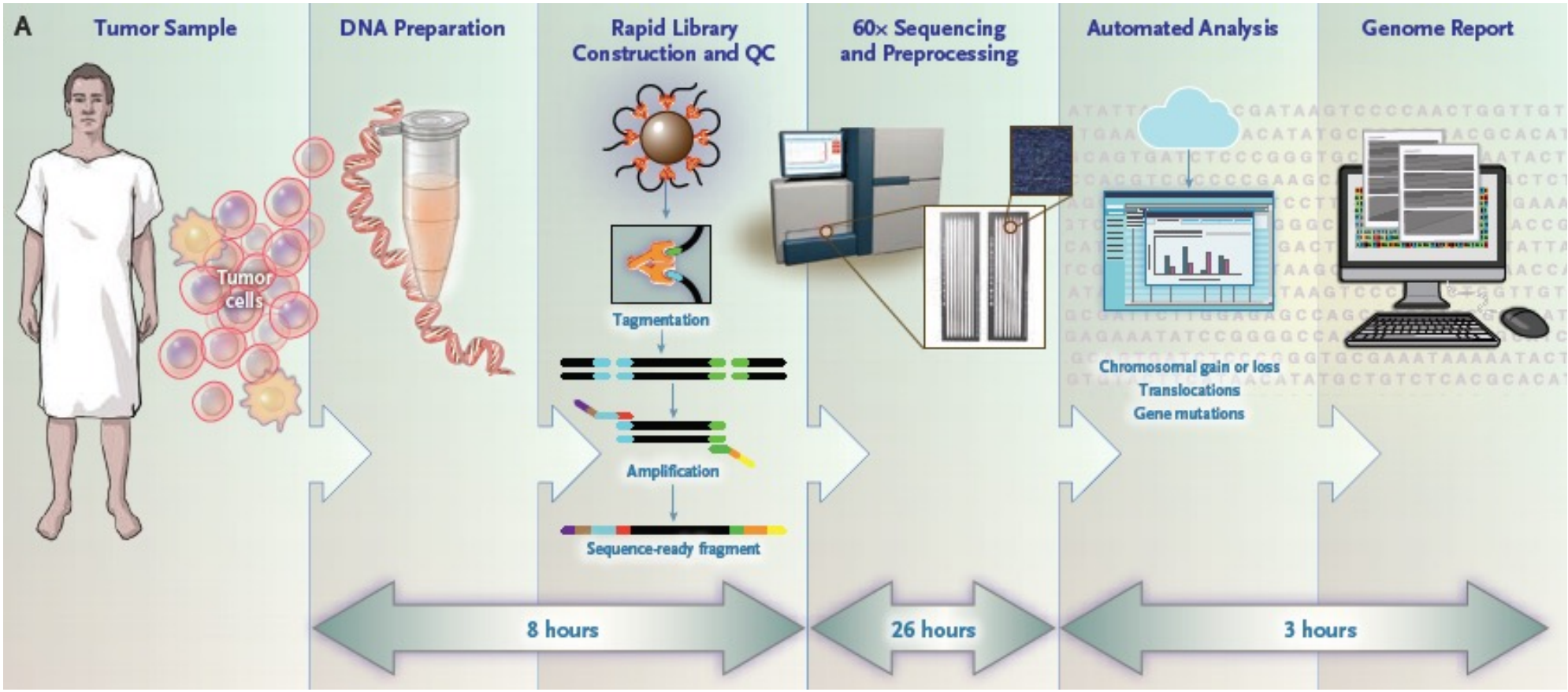
Comparison in cost and time – today

Analytic approach	Cost (USD)*	Approximate time from sampling to analytic results*
Conventional approach		
CCA	800 – 1200	Approx. 2 weeks
FISH fusion panel	Approx. 1100	Approx. 2 weeks
CCA + FISH panel	2000 – 2600	Approx. 2 weeks
NGK approach		
WTS + sWGS	1000 – 1700	Approx. 2 weeks
WTS + sWGS + LD-RTPCR	1500 – 2300	Approx. 2 weeks

Conclusions

- Very good concordance between this NGK approach and CCA for ELN defining aberrations
- Overall, NGK more sensitive for CNAs and fusions, specifically for 5q-, inv(16), t(KMT2A)
- CCA frequently report false monosomies (pseudomonosomies)
- Caution for lowly expressed fusions with this WTA strategy –
  - Deeper RNA-seq, capture, other DNA sequencing techniques (WGS), specific targeted fusions detection technique
- This NGS approach is comparable of better in cost and time compared to conventional karyotyping
- Automized bioinformatic pipeline would results in shorter processing time
- Today: WGS would be the preferred technique

Diagnostic pipeline WashU study

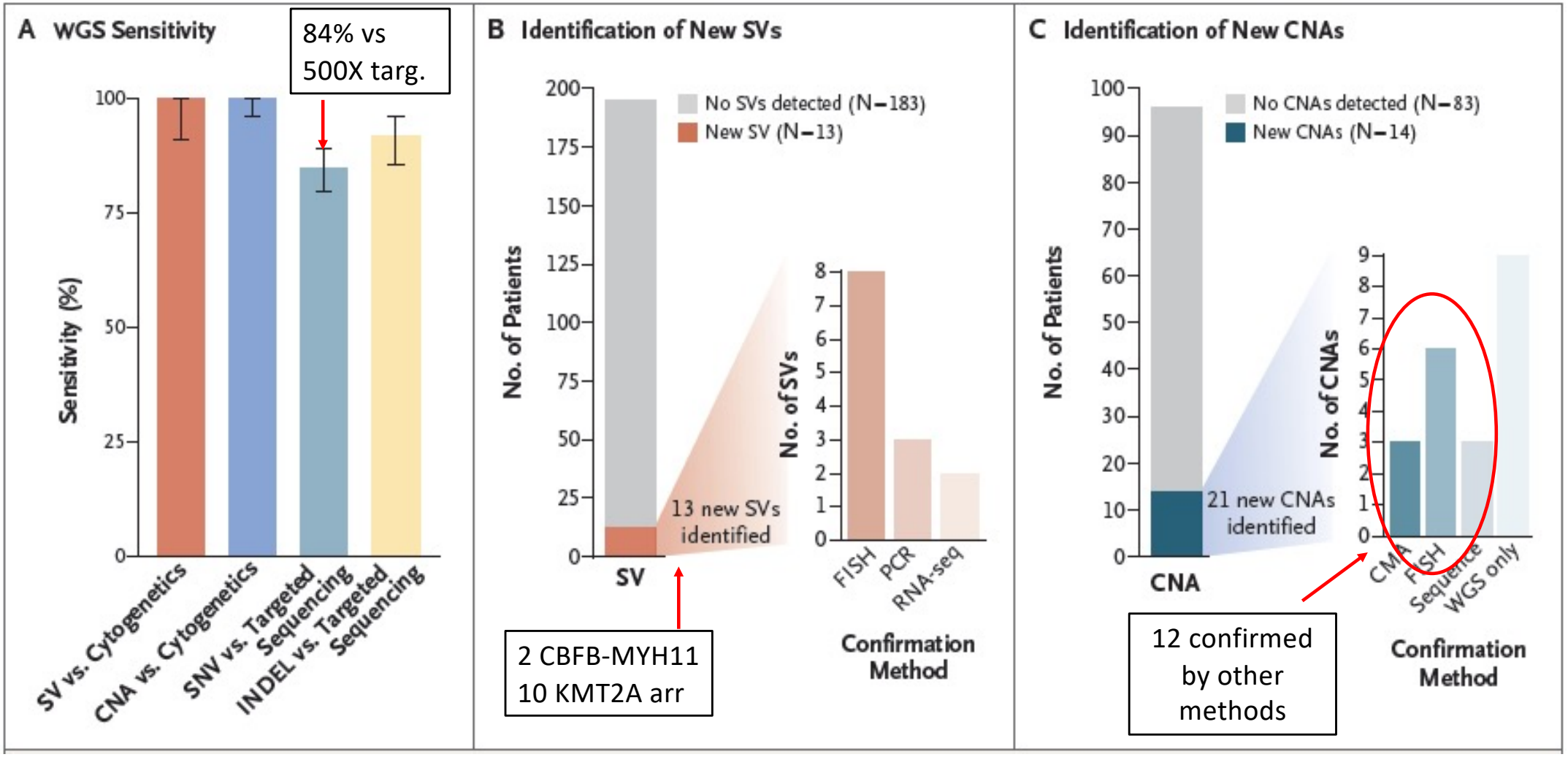


WashU study

- 147 retrospective and 117 prosoective AML and MDS samples
- AML - 107, 68 respectively

- NGS method
  - 60 X WGS
  - Automatic bioinformatic pipeline calling
    - mutations in 40 genes known to be mutated in AML
    - Copy number alterations > 5 Mbp
    - Structural variants previously described in AML (n=612)

Concordance conventional methods - WGS

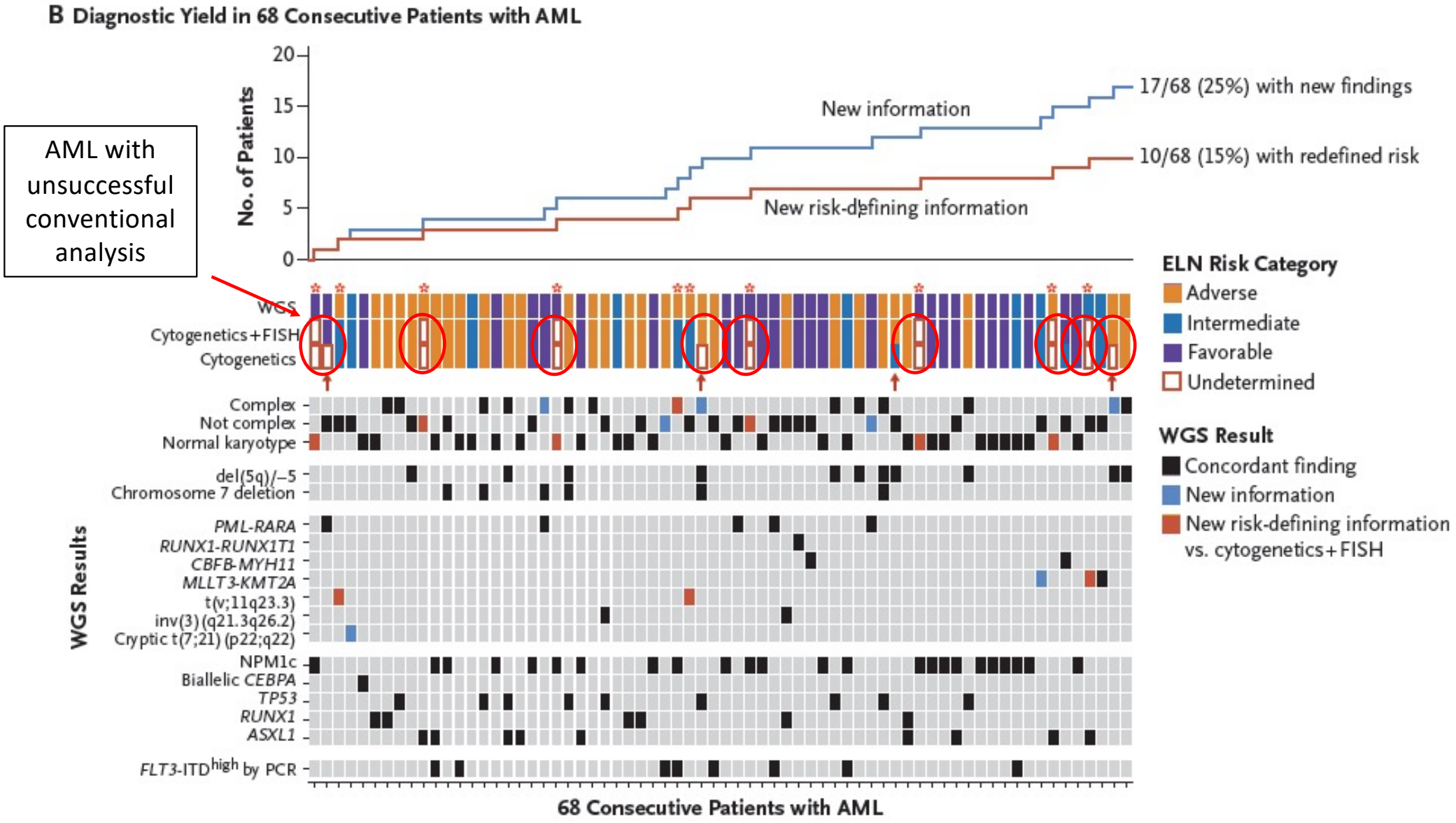


Diagnostic processing

- 117 prospective AML and MDS samples
- Weekly whole genome sequencing
- Median 5.1 days from receiving samples to results (min 3 days)
  - 2 days library preparation
  - 2 days sequencing
  - 1 day analysis

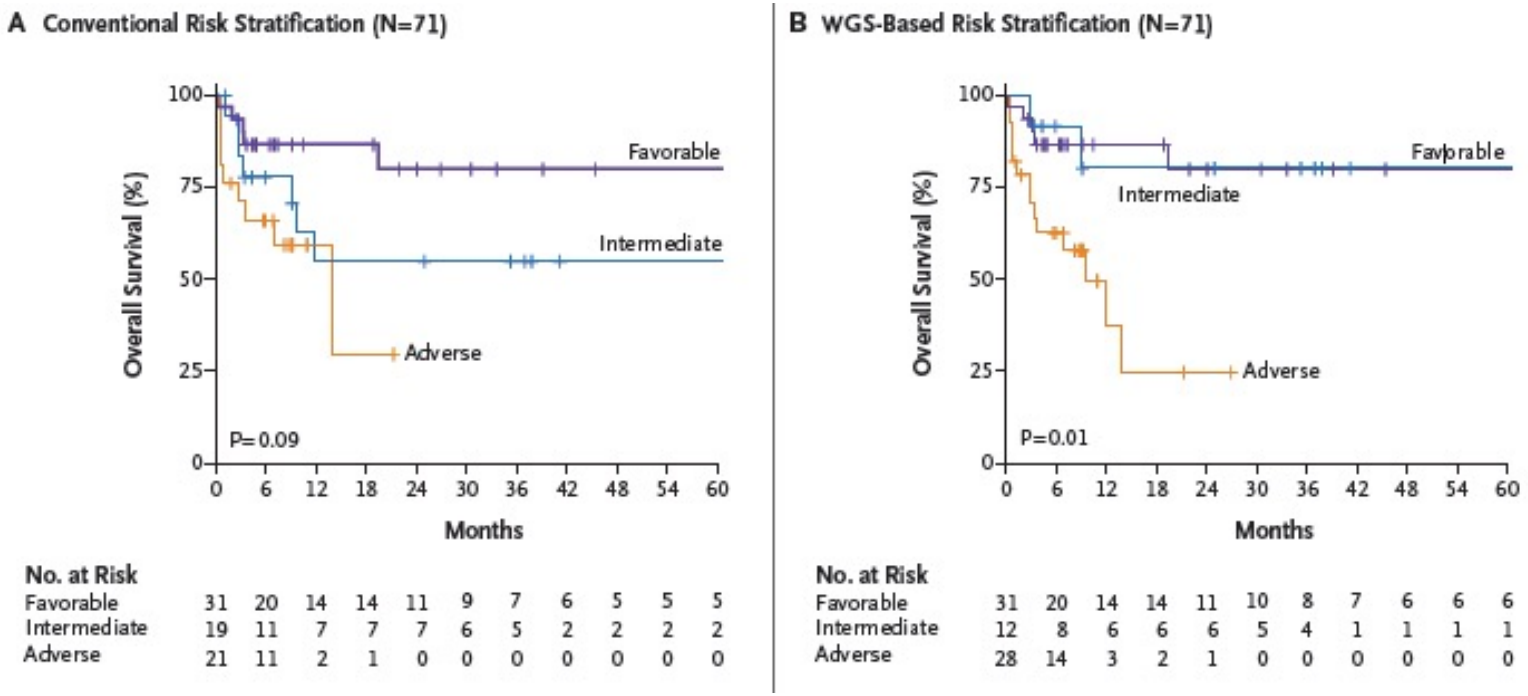
- 94% of samples produced results without manual processing of data

AML with unsuccessful conventional analysis



ELN survival analysis

- 71 AML cases, non-transplanted
- 8 (11%) changed risk group with WGS compared to conventional
- FLT—ITD was based on PCR for both approaches



Conclusions - Questions

- NGS based whole genome techniqes can accurately assign AML patients to ELN categories – and is more sensitive compared to conventional techniques
- Compared to karyotyping it is at least as good or better when it comes to cost and time lines
- How difficult and resource demanding is it to set up the NGS methods?
- What additional methods are still needed?
  - Sensitive methods for mutation calling (SNV, indels)
  - FLT-ITD allelic ratio
  - Faster methods for some results (such as PML-RARA, FLT3-ITD)?

- How do we make use of all additional information provided be genome wide analyses? – New prognostic markers – need extensive studies

Lehmann Group and Collaborators

Lehmann group

- Huthayfa Mujahed
- Anne Neddermeyer
- Anna Bohlin
- Albin Österrros
- Christer Nilsson
- Stefan Deneberg
- Anna Eriksson
- Sofia Bengtzén
- Naomi Cook
- Xiangfu Zhong
- Linda Arngården

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  - Sophia Miliara
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  - Ulf Landegren
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  - Ulla Ohlsson-Strömberg

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  - Olli Kallionemi (drug screen, systems biology)
  - Janne Lehtiö (proteomics)
  - Per Nordlund (CETSA, proteomics)
  - Thomas Helleday (drug project)
  - **Henrik Grönberg (ClinSeq)**
  - **Mattias Rantalainen (ClinSeq)**

- **Internationally**
  - Ben Tycho, New York (allele specific methylation)
  - Peggy Goodell, Houston (CTCF, DNA methylation)
  - Lars Bullinger, Berlin
  - Konny Döhner, Ulm

- **Swedish AML Group**
  - Gunnar Juliusson, Lund
  - Vladimir Lazarevic, Lund
  - Stefan Deneberg, Martin Jädersten, Stockholm
  - Lovisa Wennström, Göteborg
  - and more.

- **HERM, Karolinska**
  - Julian Walfridsson
  - Seishi Ogawa
  - Eva Hellström-Lindberg
  - Hong Qian

Cancerfonden

Vetenskapsrådet

Knut och Alice Wallenbergs Stiftelse

RADIUMHEMMETS FORSKNINGSFONDER

Stockholms läns landsting