Health Research Symposium 2024

Long noncoding RNAs in acute myeloid leukaemia
Roles for classification and prognostication

IP Ho-Wan Alvin
Department of Pathology
Queen Mary Hospital
26 November 2024

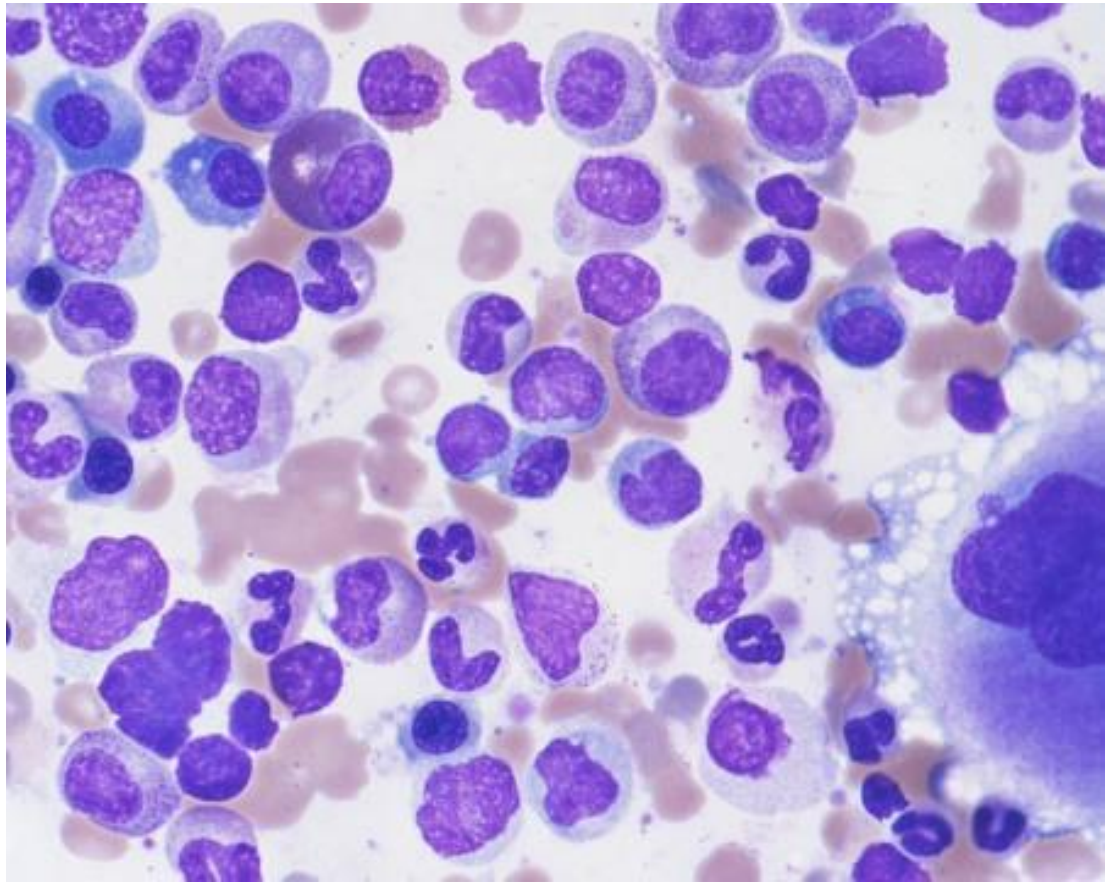
Outline

HMRF project (05160046)

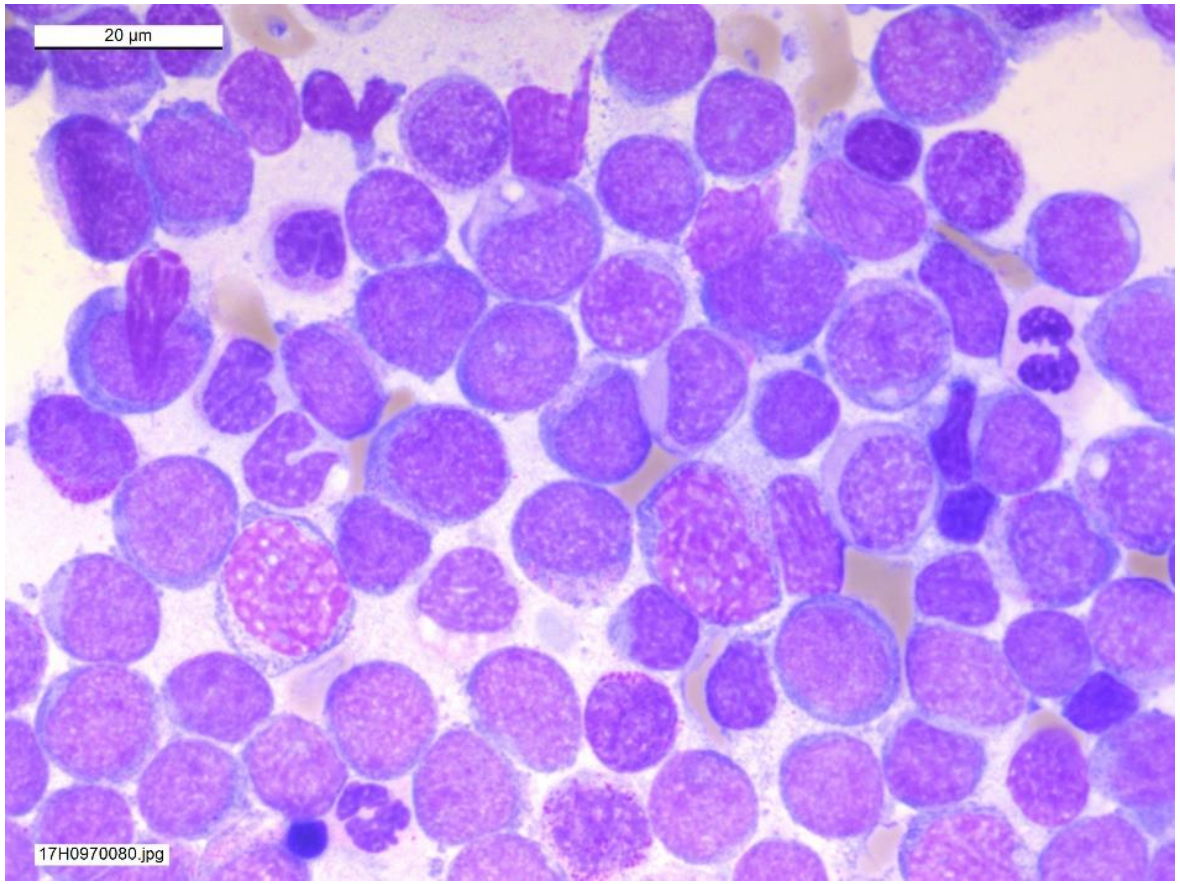
- Brief background on **acute myeloid leukaemia (AML)** and **long noncoding RNAs (lncRNAs)**
- **Prognostication** in AML
- Use of machine learning to devise **lncRNA prognostic score**
- **Validation and clinical application** of lncRNA prognostic score

Acute myeloid leukaemia (AML): Clonal disorder of myeloid stem/progenitor cells

Normal

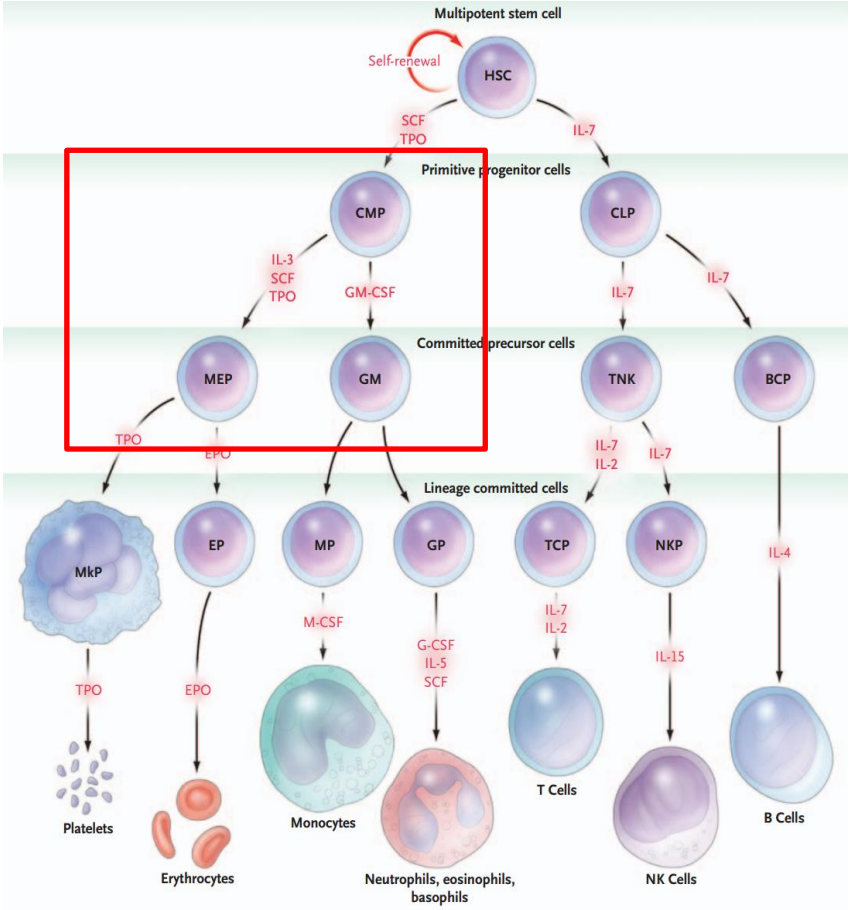


AML



Acute myeloid leukaemia
Brief introduction

- Clonal disorder of myeloid stem/progenitor cells
- ↑ proliferation
- ↓ differentiation
- **Expansion of immature/leukaemic cells** results in **severe reduction in normal blood cell production**, resulting in reduced normal blood cells in circulation
- Clinical problems: Anaemia, infections, bleeding



Kaushansky K. *N Engl J Med* 2006;354:2034-2045.

Acute myeloid leukaemia

Critical information for patient management

- **Classification**
  - Subtyping permits understanding of disease behaviour and better prediction of outcome
- **Prognostication**
  - Identify features to predict outcome
- **Treatment**
  - Select targeted therapies
- **Monitoring**
  - Identify target for post-treatment monitoring

Classification of AML

WHO 2022 Classification

Acute myeloid leukaemia with defining genetic abnormalities

- Acute promyelocytic leukaemia with *PML::RARA* fusion
- Acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion
- Acute myeloid leukaemia with *CBFB::MYH11* fusion
- Acute myeloid leukaemia with *DEK::NUP214* fusion
- Acute myeloid leukaemia with *RBM15::MRTFA* fusion
- Acute myeloid leukaemia with *BCR::ABL1* fusion
- Acute myeloid leukaemia with *KMT2A* rearrangement
- Acute myeloid leukaemia with *MECOM* rearrangement
- Acute myeloid leukaemia with *NUP98* rearrangement

Fusions

- Acute myeloid leukaemia with *NPM1* mutation
- Acute myeloid leukaemia with *CEBPA* mutation
- Acute myeloid leukaemia, myelodysplasia-related

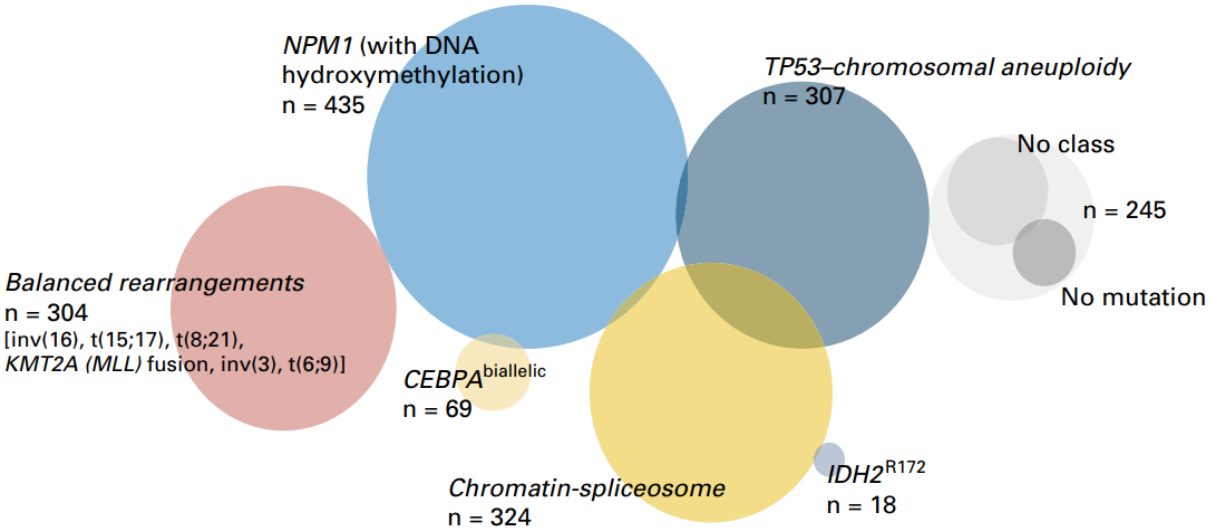
Mutations

- Acute myeloid leukaemia with other defined genetic alterations

Acute myeloid leukaemia, defined by differentiation

- Acute myeloid leukaemia with minimal differentiation
- Acute myeloid leukaemia without maturation
- Acute myeloid leukaemia with maturation
- Acute basophilic leukaemia
- Acute myelomonocytic leukaemia
- Acute monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia

Morphology



Khoury JD et al. *Leukemia* 2022;36:1703-1719.
Bullinger L et al. *J Clin Oncol* 2017;35:934-946.

Prediction of clinical outcome in AML

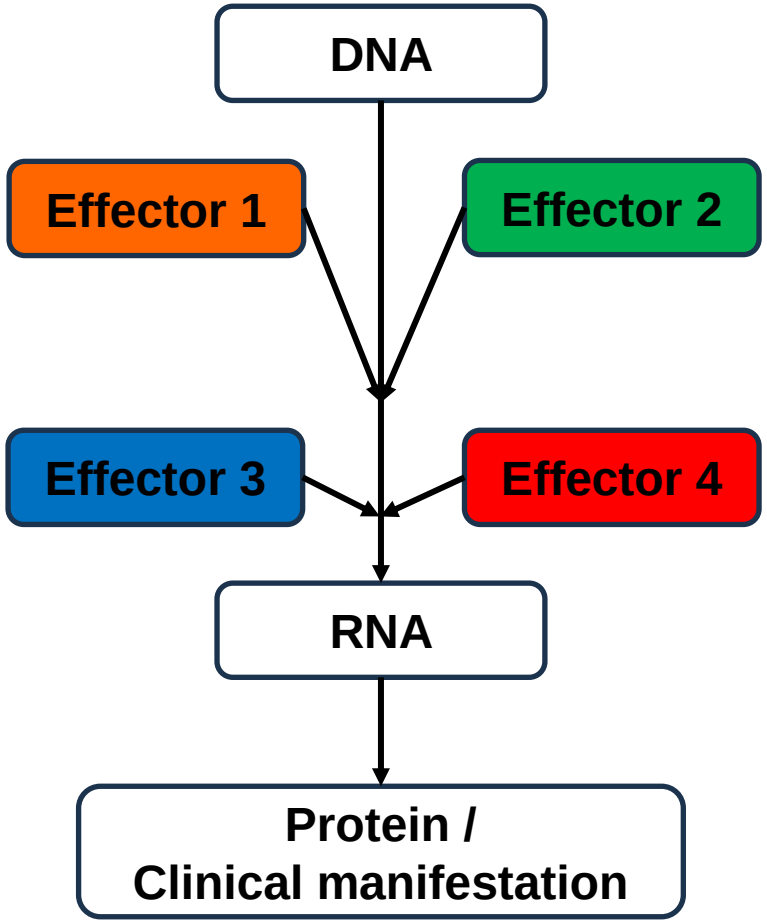
ELN risk classification

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM*(*EVI1*) • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*‡‡ • Mutated *TP53*[a]

- Latest addition of chromatin/spliceosome gene mutations in the adverse risk group has enabled prognostication in 15-20% more AML patients
- Is this optimal in predicting patients' outcome?

Döhner H et al. *Blood* 2022;140:1345-1377.

Genome vs. Transcriptome (DNA vs. RNA)



- DNA: Only detects genomic variants (spelling mistakes)
- RNA: Final common pathway that captures more effectors (or “epigenomics”)

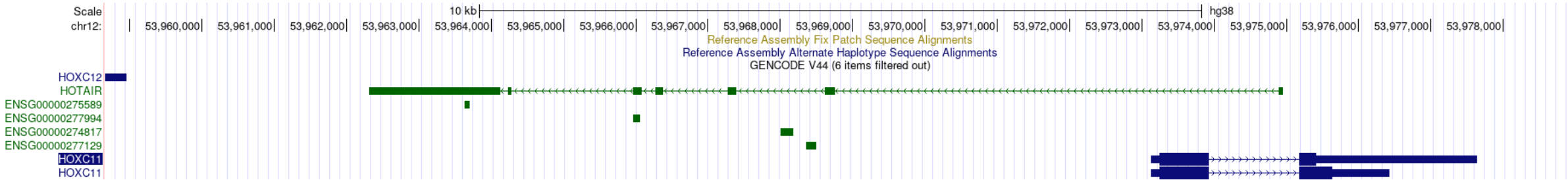
ATGCTGCATCGATCGATCGATCGATCGTACTACGATCGATCGATCGATCGATTGTGTGCA
TCGTACGACTGATCGATCGATCGTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCTAGCT
AGCTAGCTAGCATCGATCGATCGATCGATCGATCGTACGATCGATCGATCGATCGTACGA
TCGATCGATCGATCGATCGTACGATCGTACGTACGTACGTACGTACGTACGATCATCGAT
CGATCGATCGATCGATCGATCATCGTAGCATCACAACACATCGATCGTCGTACATCATCA
TCATGCTAGCTAGCTAGCTAGCTAGCTAGCTGATCGATCGTACGTAGCATGCTACGTAGC
TAGCTAGCTAGCTCGTACGTAGCTACGATCGACATCATCGTACGTAGCTACGTACGTACG
TAGCTACGTACGTACGTACGTAGCACCACAATCGATCGTAGCTAGCTAGCTGTACGTAGC
TAGCTAGTCGTAGCTAGCTACGTAGCTACGTACATGCAGTCAGTCAGTCGATATGC

Holistic picture of the human pan-genome

https://blog.createandcraft.tv/layered-papercut-art-template/

Long noncoding RNAs

Very brief introduction

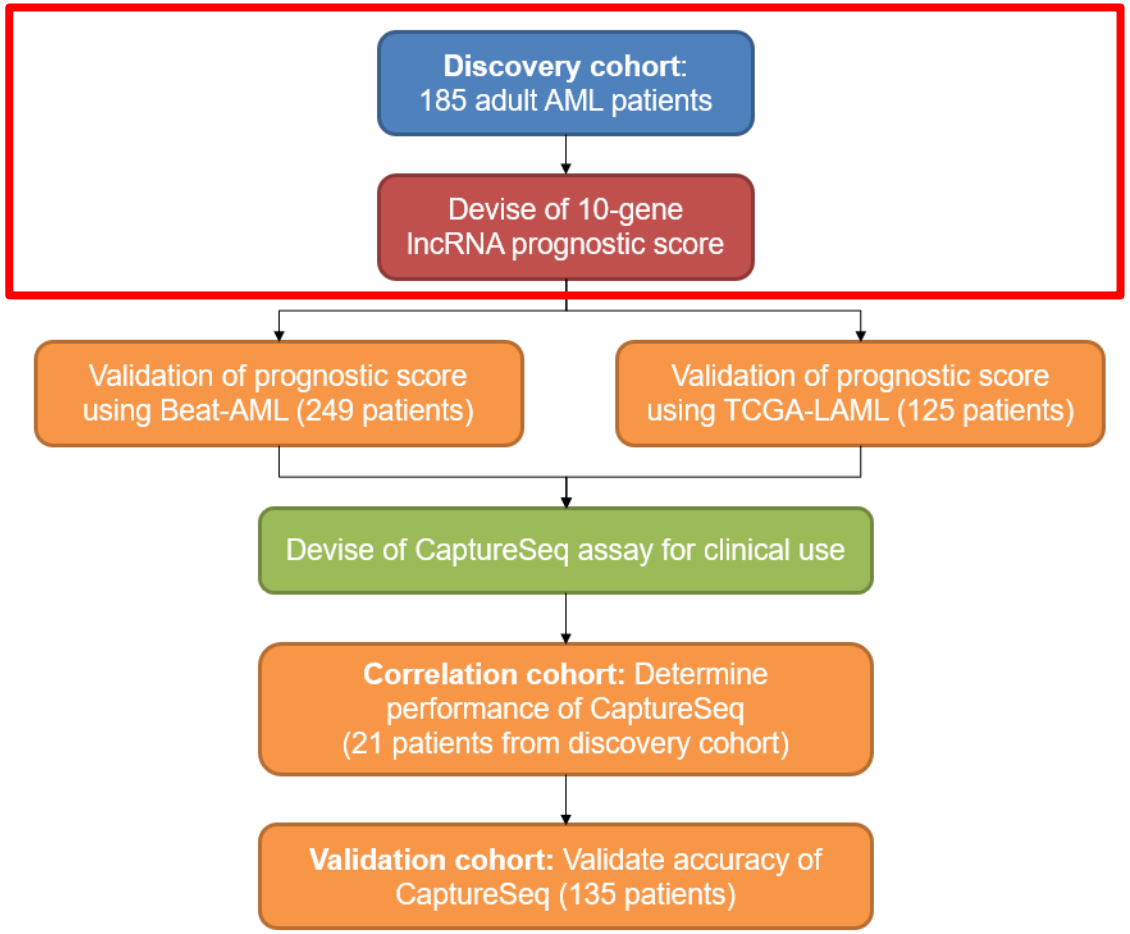


- Elements of the transcriptome
  - Messenger RNAs (protein-coding genes): 2% of human genome
  - Long noncoding RNAs (lncRNAs)
  - And many more RNA subtypes…

- lncRNAs: Transcripts longer than 200 nucleotides that do not appear to have a protein-coding sequence
- Over 100,000 lncRNAs recorded in human (cf. ~20,000 protein-coding genes)

Long noncoding RNAs in AML
Experimental design

- Project period: 2018-2022
- 185 adult patients with newly diagnosed AML for deep total transcriptome sequencing (between period of 2007 to 2018)
- Median follow-up: 417 days
- Detect only established lncRNAs to study clinical outcome



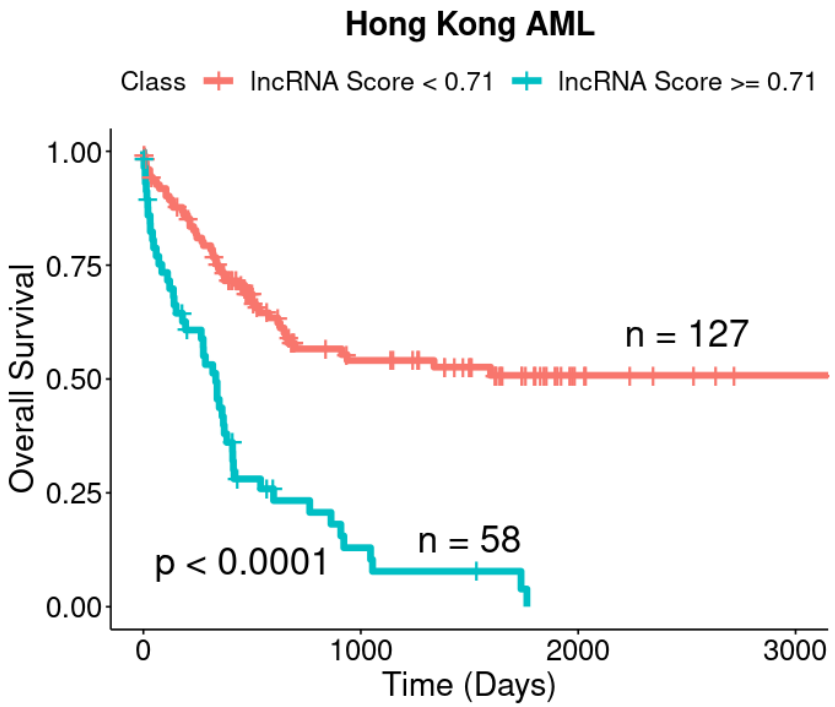
Long noncoding RNAs in AML
Classification

- Unsupervised clustering
- Classify established AML subtypes largely in accordance with their diagnostic categories
- “Blocks of colours” imply good classification of AML subtypes using lncRNAs

Long noncoding RNAs in AML

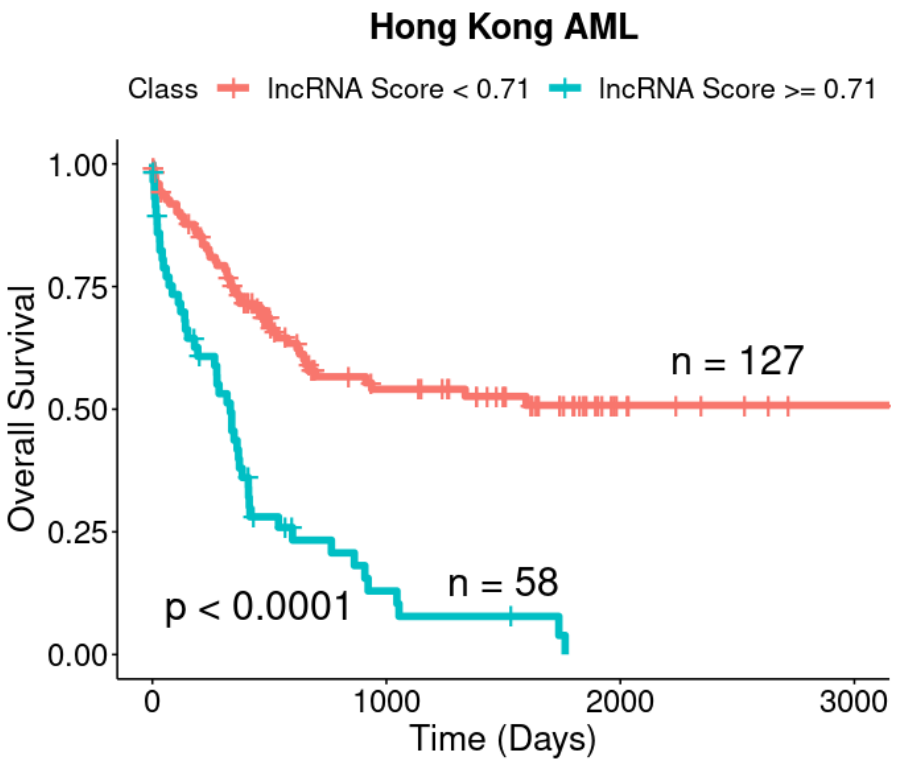
Identification of prognostic lncRNAs

- Machine learning: Lasso regression
- Identified 10 lncRNAs
- lncRNA prognostic score calculated for each patient:
  - Multiply 10 lncRNA expression level with their corresponding weighted coefficient from Lasso
  - Linearly combining their products



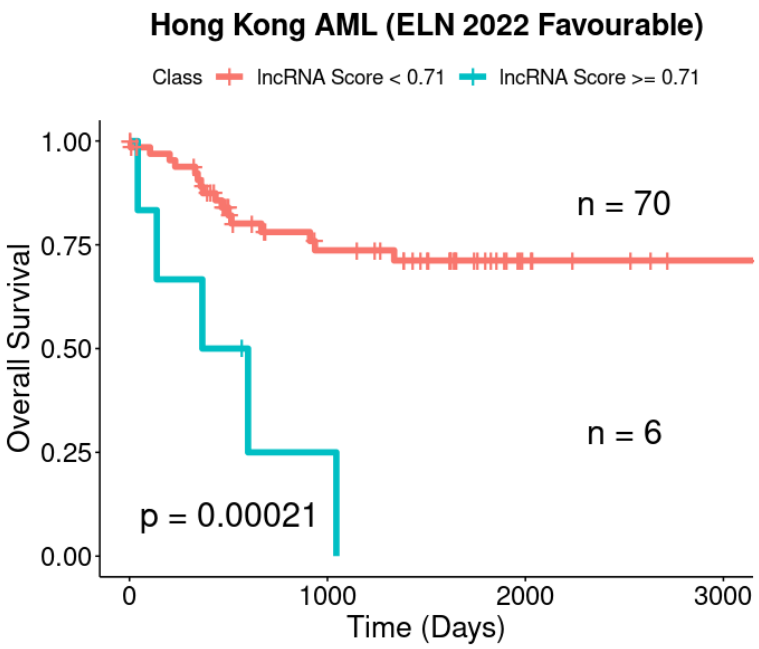
Long noncoding RNAs in AML

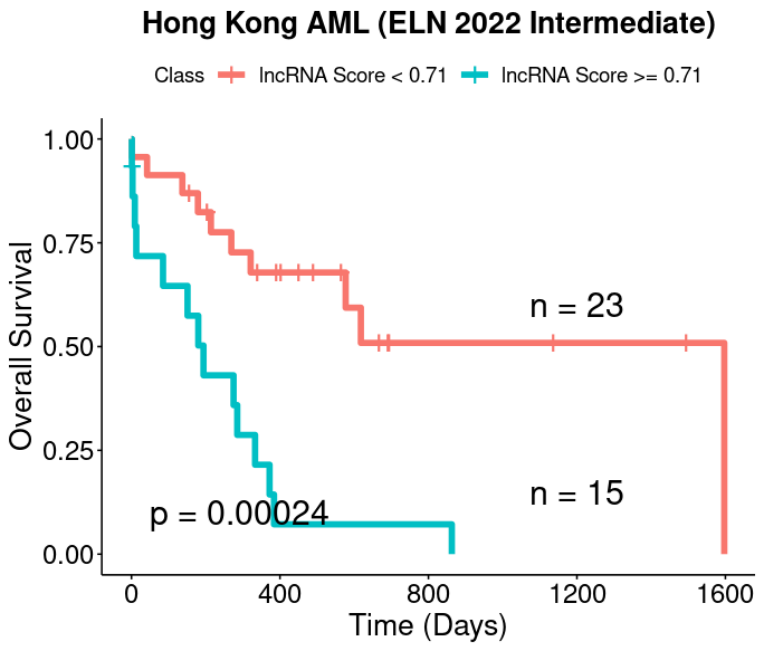
Multi-variable analysis of prognostic effects in HK cohort

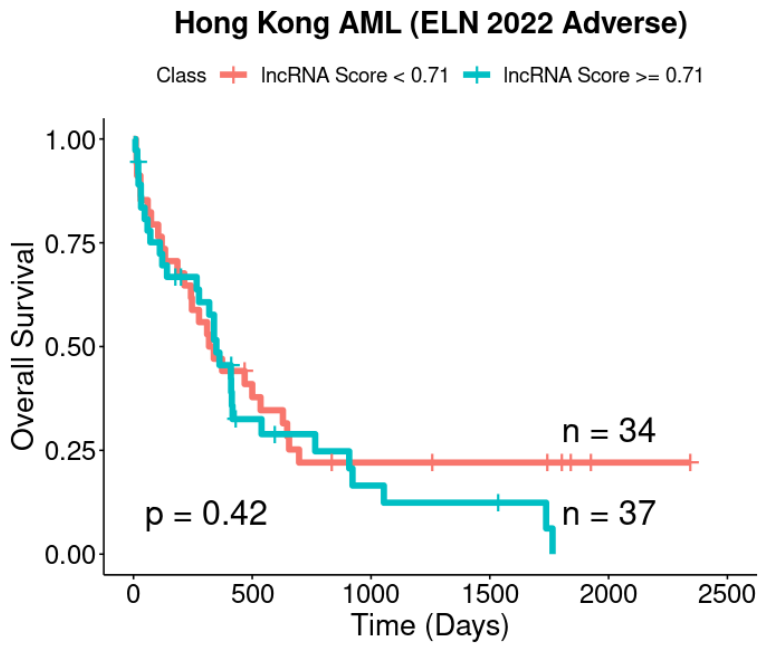


Prognostic value of lncRNAs in AML

Interactions with current prognostic system







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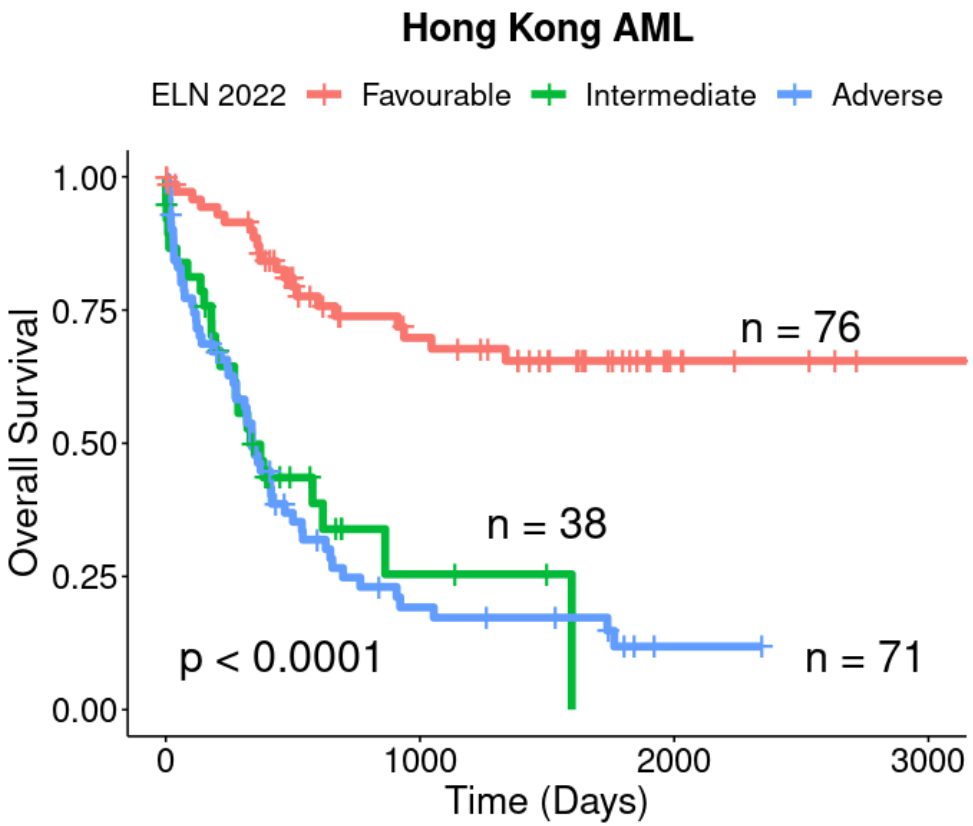
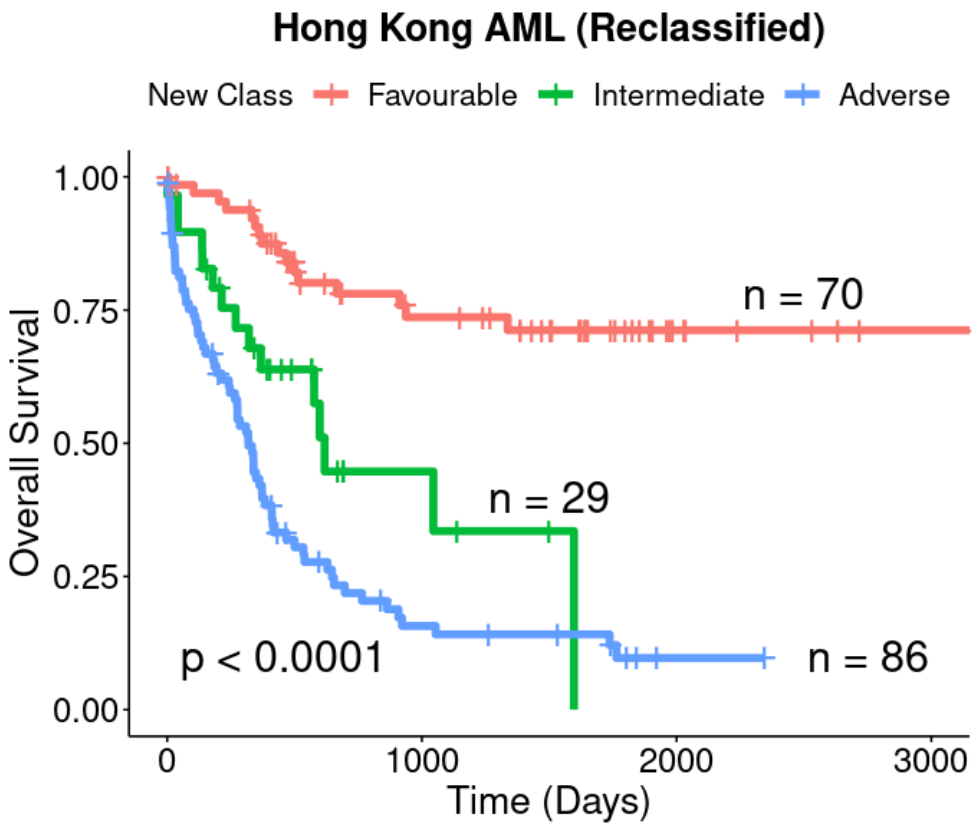




Validation of 10-lncRNA score

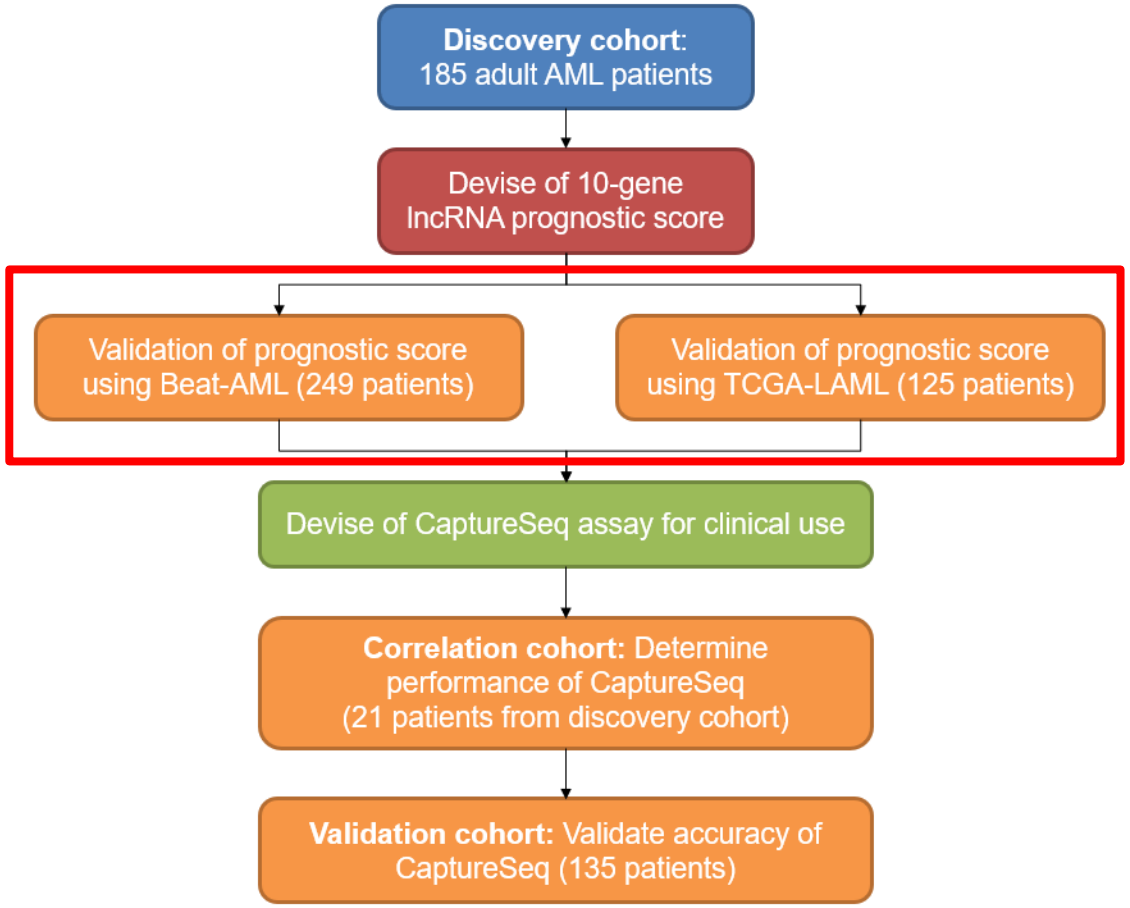
Role of external data sets

- Two well-established data sets with available
  - Transcriptome data
  - Survival data

- The Cancer Genome Atlas (TCGA)
- BeatAML

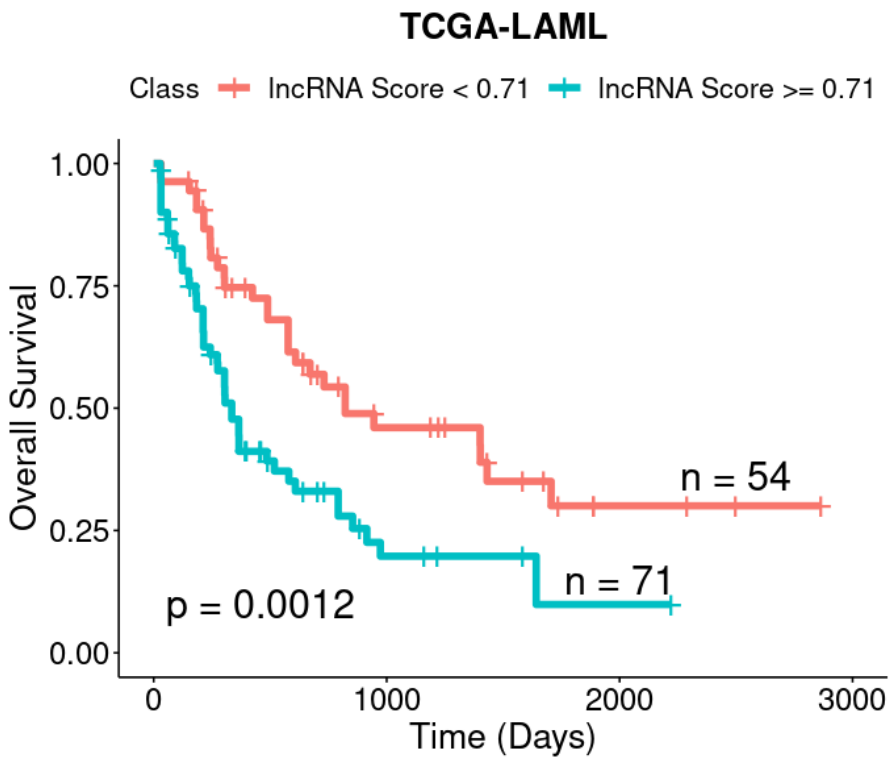
- Analysed the data in **identical manners** as the discovery cohort

- Observe whether the 10-lncRNA score retains prognostic significance on multi-variable analysis



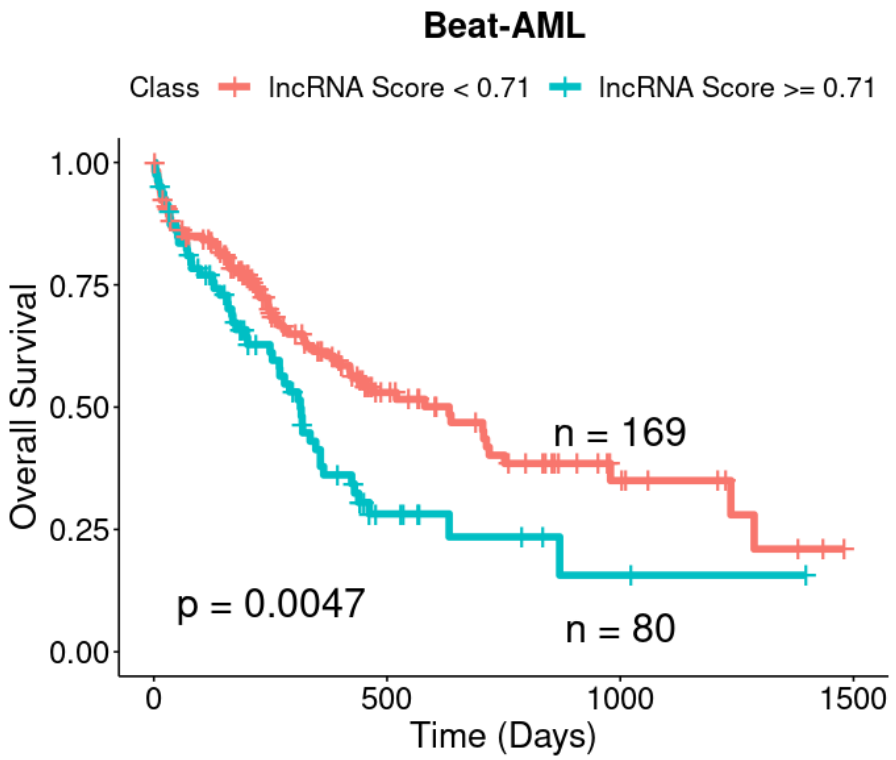
Validation of 10-lncRNA score

Multi-variable analysis in TCGA cohort



Validation of 10-lncRNA score

Multi-variable analysis in BeatAML cohort

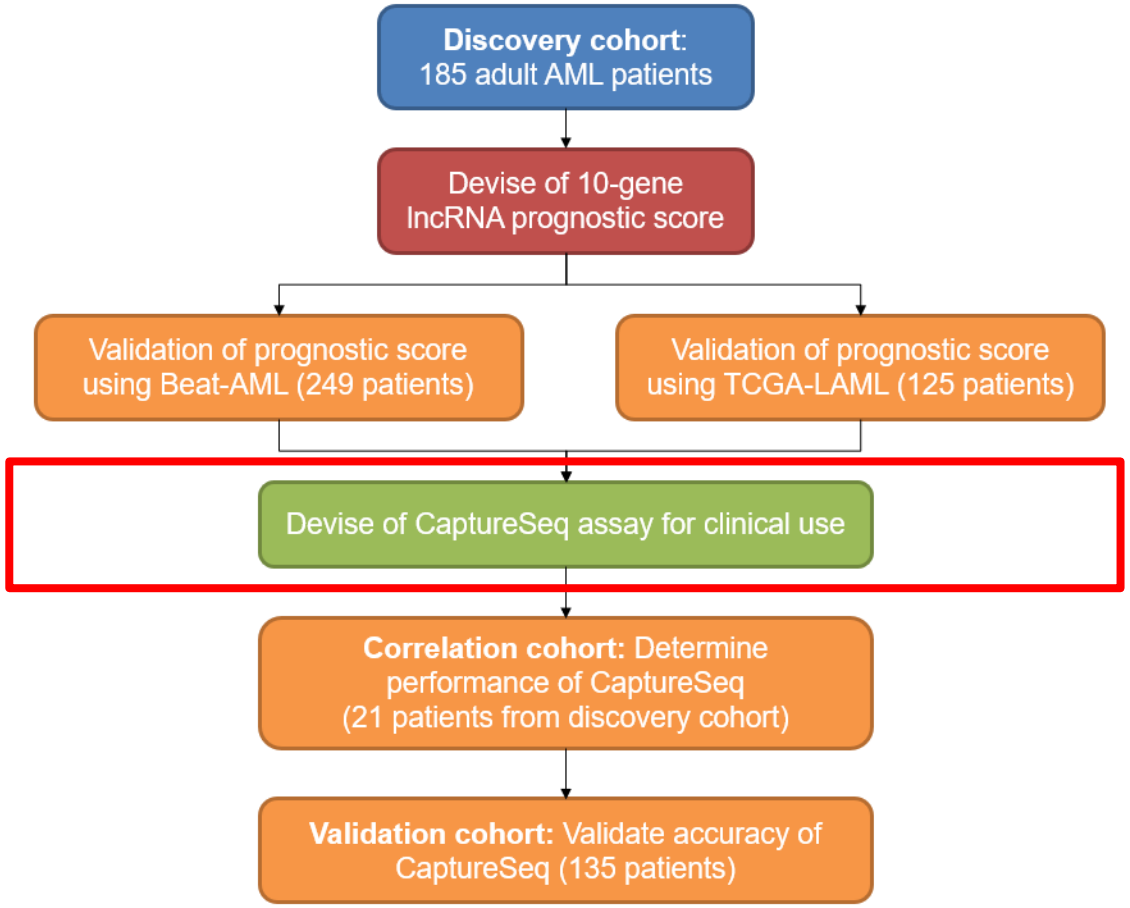


Clinical translation of research findings

Considerations for clinical applications

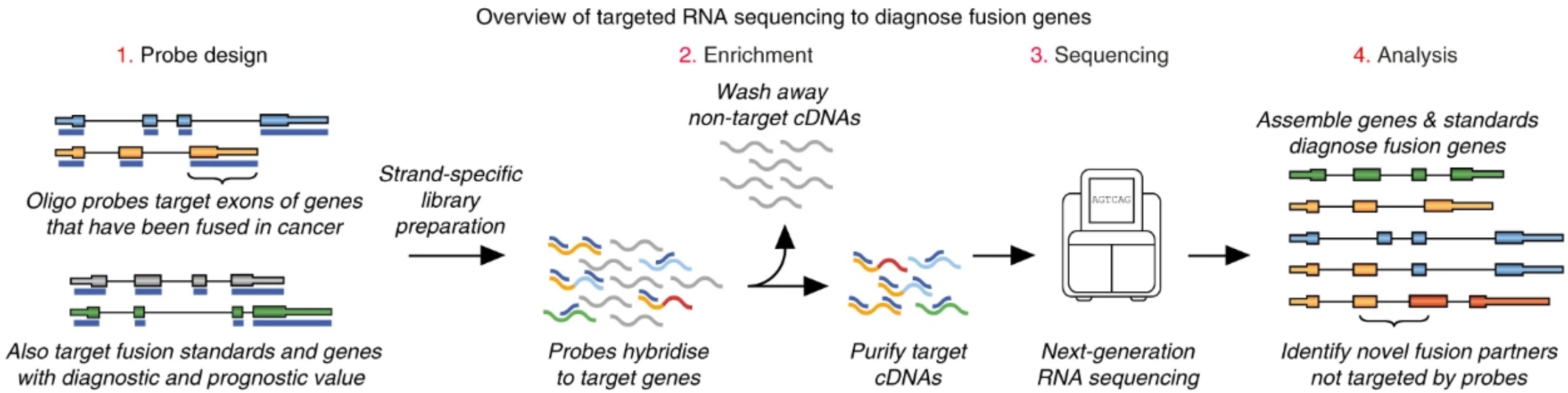
- Total transcriptome sequencing
  - Relatively expensive
  - Substantial portion of data may not have immediate clinical utility

- Enrichment of targeted genomic regions (CaptureSeq)
  - Cheaper
  - Targeted but more focused (more sequence reads to cover targeted regions)
  - Higher sensitivity



Clinical translation of research findings

Design of CaptureSeq panel for leukaemias

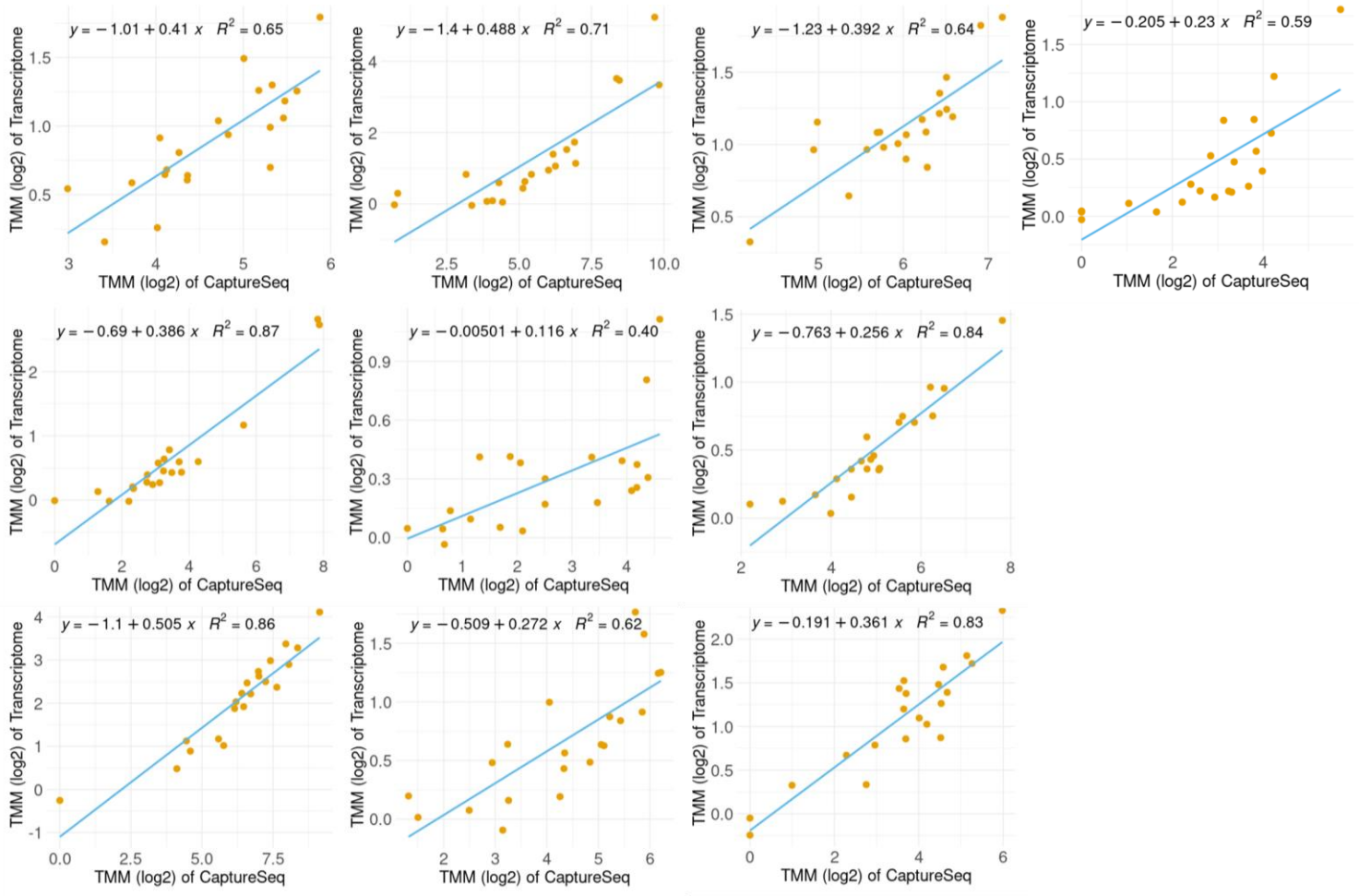


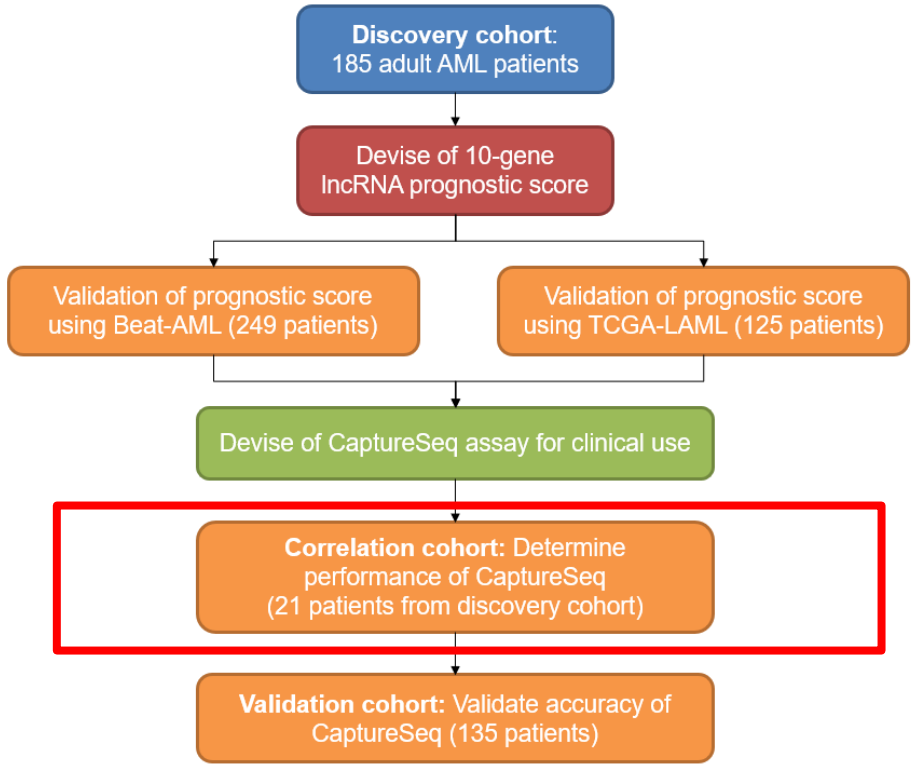
- Genes involved in **fusion** in leukaemias
- Genes for **expression profiling**: Coding genes and the 10 lncRNAs

Heyer EE et al. *Nat Commun* 2019;10:1388.

Performance of CaptureSeq panel

Correlation of 10 lncRNAs between transcriptome and CaptureSeq

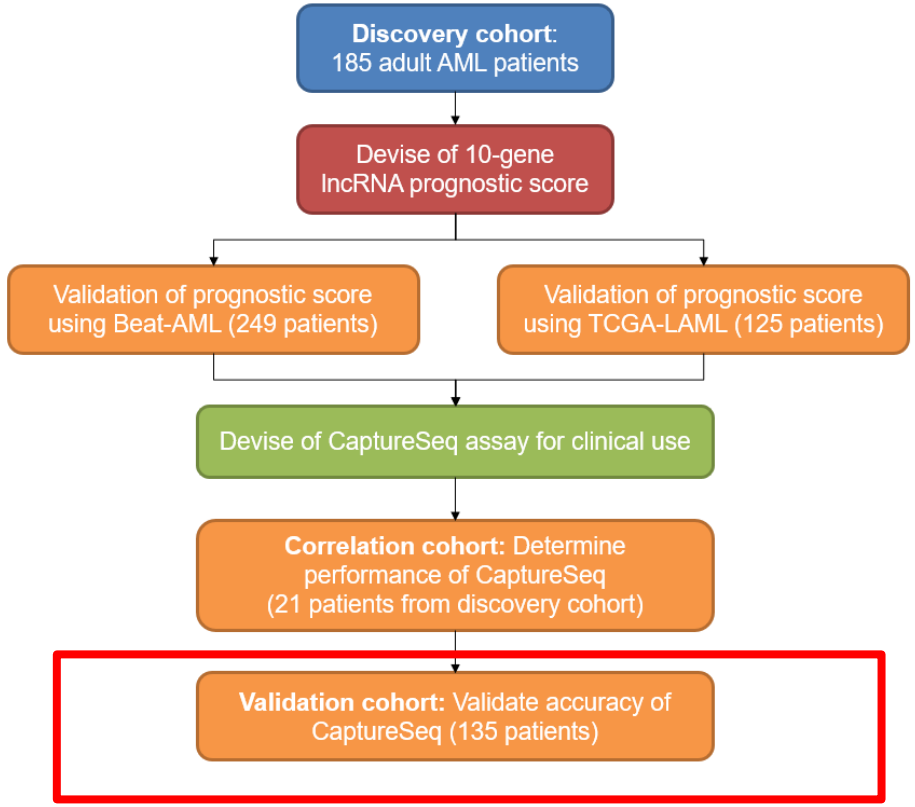




Validation of CaptureSeq panel

Independent prognostic evaluation in 135 patients

- Period: 2019-2022
- 135 consecutively recruited newly diagnosed AML patients
- Median follow-up: 335 days
- Comparison between validation cohort vs. discovery cohort
  - Older patients, e.g. 40% vs. 17% over age 70
  - More adverse risks patients, 50% vs. 38%



Validation of CaptureSeq panel

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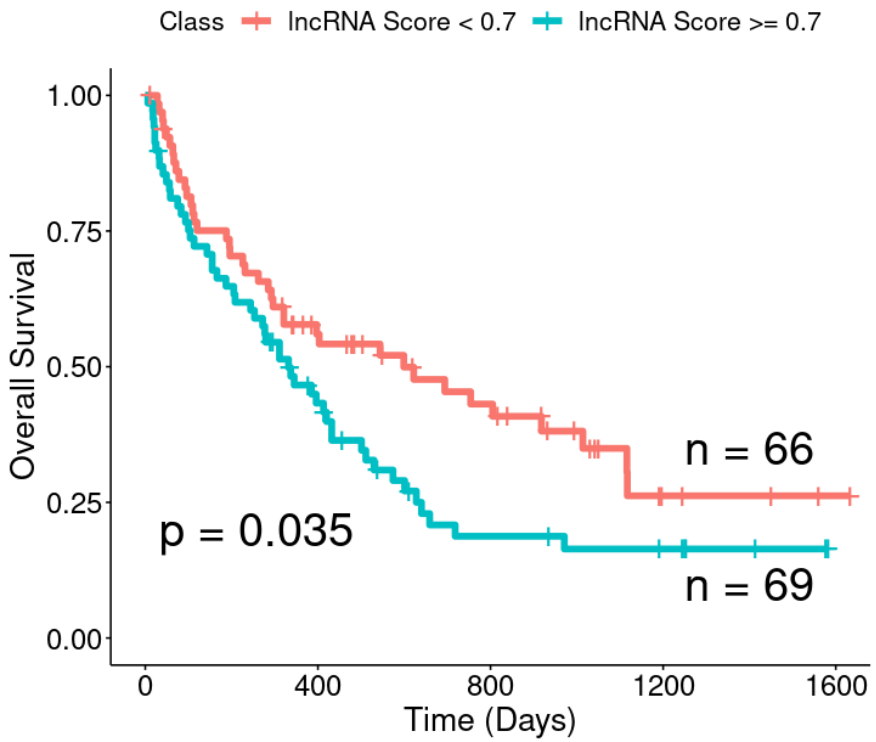
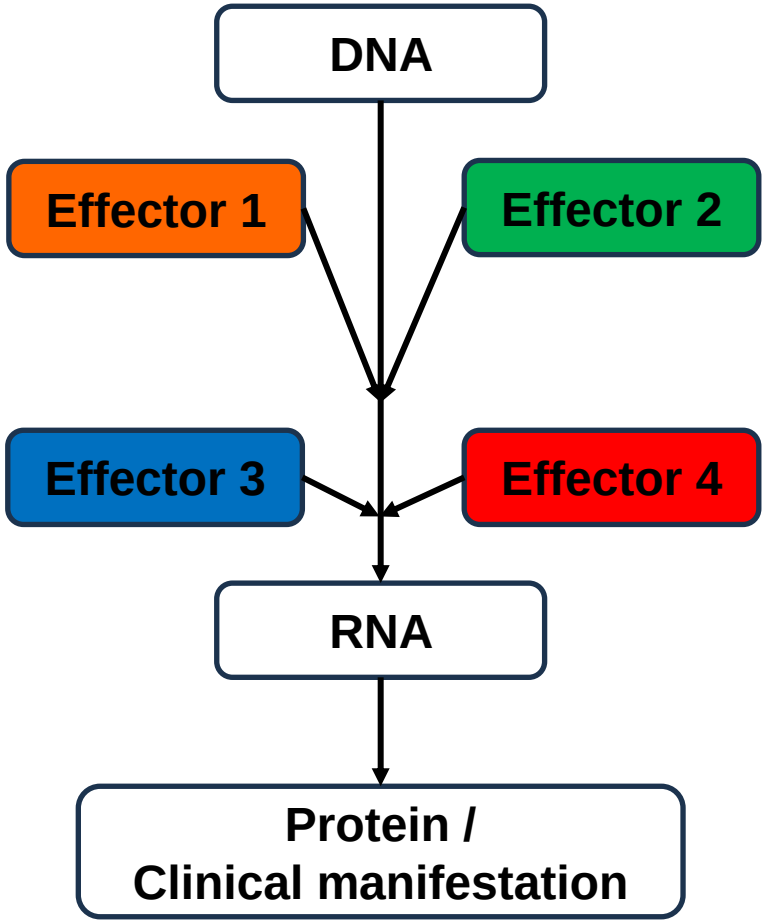


Project Summary

- Use of machine learning to identify a 10-lncRNA prognostic score
- The lncRNA prognostic score can reproducibly predict clinical outcomes of AML patients, with independent effects from the currently established prognostic parameters
- Rigorous validation of the lncRNA prognostic score using large public data sets
- CaptureSeq assay is devised for clinical translation and represents a viable option for refinement of established prognostic parameters in AML
- Substantiate the clinical utility of transcriptomics in informing the practice of precision medicine in leukaemia patients

Healthcare Implications of Project

- This project provides proof-of-concept that RNA sequencing contributes to unique information to inform clinical management
- **Genome (DNA) sequencing** provide 1st dimension of information
- **Transcriptome (RNA)** provides an indispensable 2nd dimension, i.e. functional genomics
- Cancer classification is incorporating transcriptomic information
- Prime time to strengthen our efforts in functional genomic investigations to harness **multi-omic information** to empower practice of precision medicine



Acknowledgement

- Cytogenetics and Genomics Laboratory, QMH
- Prof. Jason WH Wong, SBS, HKU
- Prof. SY Leung, SClinMed, HKU
- Prof. Anskar YH Leung, SClinMed, HKU
- Prof. H Sun, Chemical Pathology, CUHK

- Fundings
  - Health and Medical Research Fund, Health Bureau, HKSAR Government
  - Hong Kong Blood Cancer Foundation
  - Centre for Oncology and Immunology under the Health@InnoHK Initiative, funded by the ITC, HKSAR Government
  - Theme-based Research Scheme (T12-702/20-N), University Grants Committee, HK