

WHITEPAPER

FROM SEQUENCING READS TO RELIABLE MOLECULAR EVIDENCE

How Altum Sequencing's **Bioinformatics Department** supports quality-controlled ctDNA monitoring and longitudinal research — from patient-specific biomarker discovery to cohort-level evidence in follicular lymphoma.



171

Patients
Expanded FL
research cohort.

151

Follow-up samples
Published cohort,
54 patients.

95%

Trackable
In available lymph
node samples.

2×10^{-4}

Analytical target
Published sensitivity
threshold.

1. The Scientific Layer Behind Every Molecular Result

Bioinformatics is not a final processing step.

A sequencing run can generate millions of reads. Yet data volume alone does not create molecular evidence. Between raw sequencing output and an interpretable result lies a sequence of scientific decisions: which variants are biologically meaningful, which signals are technically reliable, whether a sample meets quality requirements, and how molecular changes should be interpreted over time.

At Altum Sequencing, Bioinformatics is an **integrated scientific function** connecting laboratory processes, molecular biology, computational analysis, quality control and clinical context. The team combines bioscience training with programming and statistical skills, allowing it to understand not only how to process genomic data, but also what those data may mean biologically.



WHEN THE NUMBERS LOOK RIGHT BUT THE BIOLOGY DOESN'T

*An amplicon dropout, a noisy locus, discordance between biomarkers, or a trajectory that does not fit the expected clinical course can reveal a **laboratory issue, a limitation of the assay, or a new biological observation**, even when metrics appear technically acceptable.*

What Bioinformatics makes possible:



Biomarker discovery

Patient-specific biomarker discovery and prioritization.



Standardized processing

Processing, annotation and quantification of sequencing data.



Multi-level quality control

QC at sample, amplicon, biomarker and result level.



Longitudinal interpretation

Visualization and interpretation across treatment time points.



Cohort-level analysis

Linking molecular data, imaging and clinical outcomes.

AUTOMATION:

- Standardized processing.
- Annotation & filtration.
- Demultiplexing.
- Quantification.
- Structure reporting.

SCIENTIFIC JUDGEMENT

- Biomarker prioritization
- Borderline results review
- Biological plausibility
- Complex locus assess.
- Longitudinal interpretation.

*The department does not simply process data. It turns **data into evidence** that can be trusted, questioned and interpreted.*

2. Following the Journey of a Patient-Specific Biomarker

From tumor profiling to longitudinal monitoring

Altum TRACKseq uses a **tumor-informed strategy**. Each patient's tumor is analyzed to identify somatic variants that can later be tracked in blood. From a Bioinformatics perspective, the assay begins before the first follow-up plasma sample is sequenced: it begins with the creation of a **patient-specific molecular fingerprint**.



Discovered & Evaluated

Baseline tumor or plasma sequencing identifies candidate somatic variants. Annotation, filtration and visual review assess whether each signal is real, relevant and suitable for monitoring.



Selected & Designed

A patient-specific set of biomarkers is chosen by balancing biological relevance, technical performance and low background noise. The corresponding mini-panel is then designed.



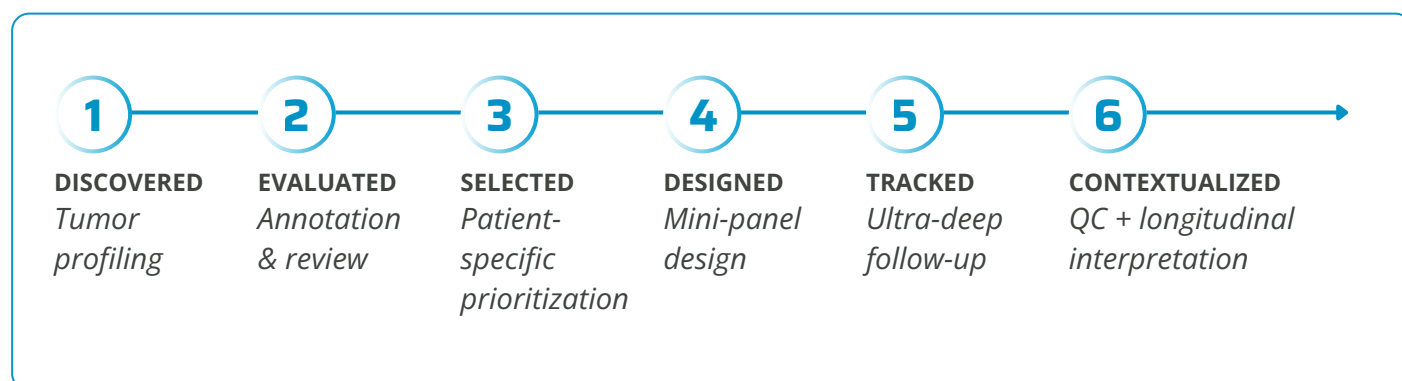
Tracked & Contextualized

Follow-up cfDNA is sequenced at ultra-deep coverage. Signals are quantified by biomarker and replicate, then interpreted against controls, QC metrics, other markers and previous time points.

EVERY BIOMARKER, THREE DECISION LAYERS

Each biomarker passes through **computational, technical and scientific decisions** before contributing to a patient result. A molecular result is built — not automatically produced.

THE JOURNEY OF A PATIENT-SPECIFIC BIOMARKER



3. Building Confidence at Every Analytical Level

Quality is an architecture, not a single threshold

In high-sensitivity ctDNA monitoring, technical noise can resemble true residual disease. Confidence therefore emerges from **multiple layers of evidence** rather than from a single variant allele frequency cut-off.



Sample quality

cfDNA input and purity influence achievable sensitivity.



Sequencing performance

Amplicon yield, useful reads and effective depth determine whether a biomarker was adequately interrogated.



Biomarker behavior

Local genomic context may create locus-specific noise or reduce usable reads.



Replicate consistency

Independent measurements reveal technical variability and support result review.



Healthy-control background

Control samples define biomarker-specific noise and detection limits.



Result classification

A signal may be positive, negative or non-evaluable depending on signal, quality and background.



WHAT A NEGATIVE RESULT REQUIRES

A negative result is meaningful only when the sample and biomarker have sufficient analytical quality to support it.

Many workflow steps can be automated, including data processing, annotation, demultiplexing, quantification and report preparation. Borderline cases, unusual loci and biological inconsistencies still require **expert confirmation**. The aim is not to automate every decision, but to standardize what should be reproducible and preserve scientific judgement where complexity matters.

4. Follicular Lymphoma as a Real-World Demonstration

From published validation to expanded longitudinal evidence

Follicular lymphoma is an informative model for molecular monitoring. It is generally indolent but recurrent, and **approximately 15–20% of patients are refractory or progress within 24 months** of first-line therapy. PET/CT remains central to response assessment, but imaging may be affected by limited sensitivity, non-specific uptake and reader-dependent interpretation.

The prospective study published in *Leukemia* in 2023* evaluated liquid biopsy MRD by ultra-deep sequencing together with PET/CT in **84 patients**. Trackable mutations were identified in **95% of available lymph node samples** and **80% of baseline plasma samples**. Patient-specific biomarkers were subsequently assessed in **151 follow-up plasma samples from 54 treated patients**.

84

Patients

Published
prospective cohort

151

Follow-up samples

From 54 treated
patients

95%

Trackable

In lymph node
samples

2×10^{-4}

Analytical target

Published sensitivity

The published work showed that molecular positivity at interim and end-of-treatment time points was associated with **increased progression risk**. When LiqBio-MRD and PET/CT results were combined, the published analysis identified POD24 with **88% sensitivity and 100% specificity** among patients with concordant results.

RESEARCH PROGRAM EXPANSION

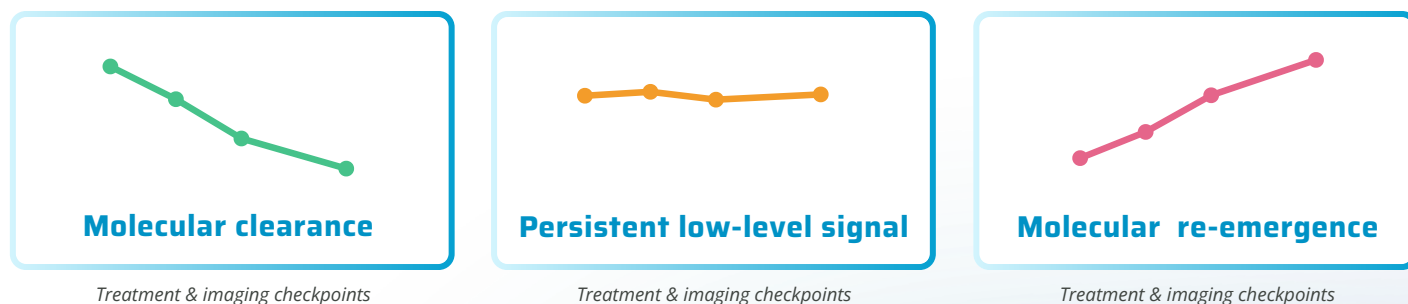
The research program has since expanded to **171 patients**. The new dataset supports broader longitudinal and patient-level analyses, but updated sensitivity, specificity, predictive values, survival estimates and hazard ratios **remain reserved for publication**.

*Source: Jiménez-Ubieto A. et al. *Leukemia* 2023;37:659–669

5. Beyond Positive or Negative

Interpreting molecular dynamics

A positive or negative call is necessary, but longitudinal interpretation requires understanding **how the molecular signal behaves over time**. Rapid clearance, persistent low-level signal and molecular re-emergence can reflect different biological situations and should be interpreted together with treatment phase, imaging and clinical evolution.



This is where Bioinformatics creates value beyond sample processing. By connecting multiple time points, biomarkers, PET/CT assessments and clinical outcomes, the department transforms individual measurements into **patient-level trajectories and cohort-level evidence**.

Conclusion

High-sensitivity ctDNA monitoring requires more than sequencing technology. It requires a framework capable of selecting appropriate biomarkers, controlling technical noise, evaluating analytical quality and interpreting molecular changes over time.

At Altum Sequencing, Bioinformatics provides that framework. From the original 84-patient study to the expanded **171-patient follicular lymphoma cohort**, the department has supported the transition from individual sequencing reads to longitudinal disease understanding.

*Bioinformatics is not behind the scenes at Altum Sequencing. It is one of the **scientific engines** that makes tumor-informed molecular monitoring possible.*

Are you a doctor or medical professional?

Interested in learning more about Altum tech?

Curious about integrating our tech into your hospital?

