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Acute myeloid leukemia - Section 1

Early response evaluation in AML using mass cytometry

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Take home messages

- Mass cytometry is a research tool that allows sample barcoding and detection of more than 40 single cell parameters, theoretically over 100, exceeding the current limits of conventional flow cytometry.
- Simultaneous single cell investigation of composite immunophenotypes and intracellular signaling proteins, combined with scalability for detection of gene expression and mutations, provide a unique insight in blood and bone marrow cell populations when evaluating response to conventional and novel therapies.
- Early evaluation of treatment response by mass cytometry can potentially distinguish therapy responders from non-responders at the level of minute cell populations and leukemic clones, allowing identification of actionable targets for adjuvant therapy and prevention of disease relapse.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous and aggressive blood cancer where diagnosis is based on morphology, immunophenotyping and genetics. Genetics is increasingly guiding classification, risk stratification and selection of therapy in AML.[1] AML blasts reflect the pattern of antigen expression of hematopoietic differentiation, but with distinct abnormal immunophenotypic profiles. Therefore, immunophenotyping is also central in diagnostics and determination of minimal residual disease (MRD).[1,2]

Mass cytometry (CyTOF) is a fusion of two technological platforms: flow cytometry and elemental mass spectrometry.[3] CyTOF is currently a research tool in hematology, and a substantial effort is needed if CyTOF should approach a standard of care in diagnostics, illustrated by the impressive long-term work of the EuroFlow Consortium on harmonizing immunophenotyping. In CyTOF, antibodies conjugated to stable heavy metal isotopes which are quantified using time-of-flight mass spectrometry. The advantage of CyTOF lies in the ability to detect isotopes of different atomic mass with very high accuracy and minimal signal overlap. CyTOF has, therefore, opened up a new avenue for resolving the clonal complexity of AML in a hematopoietic continuum.[4]

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Current state of the art

Early response evaluation in AML

AML patients fit for intensive treatment are initially treated as one-size-fits-all with chemotherapy, with an addition of targeted therapy based on mutational data, for example, inhibitors of FLT3 kinase or IDH1/2. Current clinical practice include morphological response evaluation of the first cycle of induction therapy on day 14 to 17, and mandatory before the second course when peripheral blood values should be normalized.[1] Evaluation of MRD is commonly performed by flow cytometry or quantitative genomics after the first or second course of chemotherapy, preferably from bone marrow, even if peripheral blood is suggested.[2,5] Thus, often 2 months pass before MRD evaluation is performed. We suggest that an earlier determination of therapy response by flow cytometry or CyTOF could provide advantages by allowing personalized therapy optimization in suboptimal responders. Meaningful early response evaluation is also supported by studies performing bone marrow examination at day five post induction chemotherapy[6] and the explorative PET-CT imaging 2 days after treatment onset.[7]

Studies have also shown that proteins and genes associated with chemotherapy resistance and cell survival are modulated only hours after start of induction therapy in AML.[8] CyTOF could prove superior to these studies investigating bulk cell populations by elucidating functional therapeutic responses in smaller cellular subsets of both leukemic and non-leukemic cells.[9,*10]

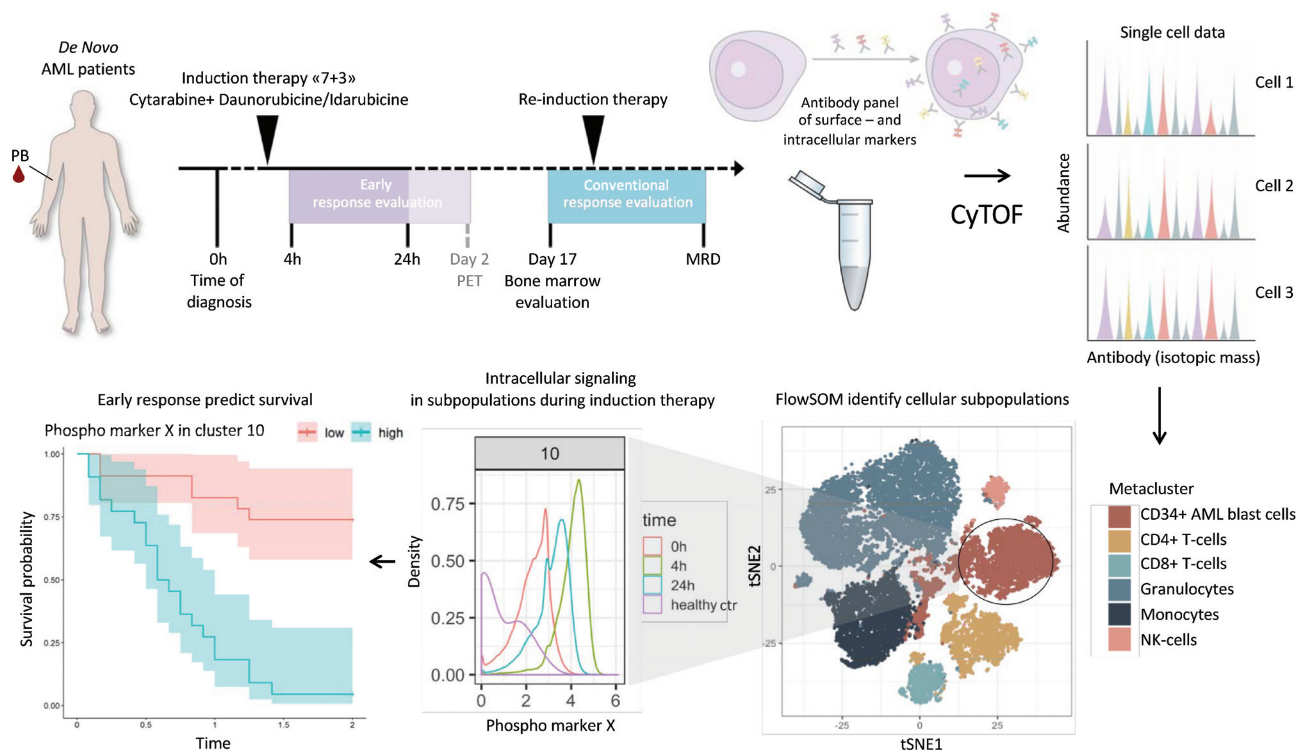

Figure 1. Flowchart for investigation of early signaling response by peripheral blood sampling from newly diagnosed AML patients during induction therapy. Peripheral blood (PB) can be collected at early time points such as 4 to 24 hours after start of therapy. Cells are then barcoded and stained with suitable antibody panels for both surface and intracellular markers of interest. CyTOF provide single cell data, and tools for data visualization and dimensionality reduction is necessary. FlowSOM is an unsupervised algorithm that applies machine learning by Self- Organizing Maps (SOM) to reveal meaningful cell clusters and detection of rare cell subsets. Changes in the intracellular signaling in metaclusters identified by FlowSOM can then be analyzed and compared to clinical parameters such as complete remission and survival. (Tislevoll BS, Fagerholt OHE, Blaser N et al. Blood; Dec 1-4, 2018. San Diego, CA. Abstract 1502).

Increased resolution combined with cellular functionality read-outs

CyTOF has previously been used to demonstrate the plasticity of immunophenotypes immediately following induction therapy in AML.[11] Additionally, the immunophenotype for leukemia initiating cells, and especially leukemic stem cells, is not uniform.[12] Identification of these heterogeneous cell populations at MRD evaluation requires high resolution of multiple surface markers, which is easily achievable in a single sample using CyTOF.

CyTOF analyses have indicated that intracellular signaling rather than surface markers may be more representative of the diseased cell function, and that this can be related to gene expression signatures and correlated to patient survival.[*13] Functional responses to environmental stimuli have been shown to reveal a broader signaling network than can be measured in the basal cell state, exposing the cells ex vivo to a panel of perturbators like growth factors, cytokines and small molecule inhibitors.[14,*15] However, we argue that by performing peripheral blood sampling followed by immediate cell fixation before and shortly after therapy onset, the therapy itself acts as an in vivo perturbator to unravel cellular responses leading to a beneficial therapy response (Fig. 1).[*16]

So far, early response evaluation by CyTOF has been performed in few clinical trials. In the chronic phase CML trial ENEST1st monotherapy with nilotinib elicited intracellular signaling responses 24 hours and 7 days after start of therapy, and when correlated with quantitative PCR of BCR-ABL1 at 18 months, the CyTOF analysis identified optimal responders within a week.[*16]

A single-cell characterization of leukemic cell intracellular responses to induction therapy in AML patients could identify cellular subsets that flag responder status of the patient, clinically relevant mechanisms of chemotherapy resistance and reveal potential novel therapeutic targets to guide personalized therapy (Fig. 1).[17] CyTOF studies have elegantly demonstrated treatment-specific responses in specific cellular subsets in AML patients, further underscoring the advantages of CyTOF in investigation of differential treatment responses in subpopulations of composite heterogeneous patient samples.[*15,*18] Likewise, CyTOF is an ideal tool to evaluate dynamic signaling responses to administration of novel targeted therapies in AML, as sample multiplexing allows for direct comparison of sequential samples from a single patient (Hellesøy M, et al HemaSphere; Jun 14–17, 2018; Abstract PS965). Increased dimensionality combined with standardization of measurements through sample multiplexing makes CyTOF a suitable tool in a clinical trial setting. CyTOF is currently not a standard diagnostic tool in hematology, but has strongly advanced the research field of single cell analyses through increased dimensionality, allowing observation of phenotypic diversity, plasticity and functional behavior in a single sample.

Future perspectives

The limited but promising use of CyTOF in multicenter clinical trials indicates that future development into routine diagnostics may be possible, providing a dimensionality that allows monitoring of cellular responses to targeted therapy. The major limitations currently include lack of validation in larger clinical trials, expensive machines (eg, approx. 3× the price of a routine

flow cytometer) provided by a limited number of vendors, high running costs (eg, argon gas approx. 15,000€/year), slow analysis throughput (eg, maximum 600 cells/s vs 25,000 cells/sec by flow cytometry) and need for advanced bioinformatic pipelines for data analysis. On the other hand, antibody conjugation and validation are predictable and robust, and a well-designed panel of antibodies is likely cheaper due to sample multiplexing (€1.5–3 vs €2–8 per antibody per sample). Sample multiplexing through barcoding allows internal standardized controls, including biological controls for antibody staining and technical controls for machine variation, which would provide important quality control assessment of samples collected in large multi-center clinical trials. The CyTOF antibody panels can be adapted for analyses of samples from patients receiving various therapy modalities, as variants of CyTOF labeling techniques allow single cell detection of mRNA expression, translocated genes and various mutational DNA features in the leukemic cell population.[*19] In summary, CyTOF could form a future diagnostic platform to replace, or at least reduce, the manifold techniques currently used for diagnosis and follow-up monitoring. However, to ascertain quality control of experiments in a clinical setting, it is absolutely essential that standard bioinformatic pipelines are established to handle the complex analyses of such multidimensional data.

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