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EHA Perspectives on Emerging Technologies in Hematology

Highlights from EHA2024 Hybrid Congress held in Madrid, Spain
July 2024

Welcome

On behalf of the European Hematology Association (EHA), we are delighted to present the EHA2024 Scientific Congress Report, titled “EHA Perspectives on Emerging Technologies in Hematology.”
This report has been developed as a pilot initiative for future EHA congresses, providing our community with a concise overview of the innovative work showcased at EHA2024. This inaugural edition serves as an essential summary of scientific information and breaking news on the latest technological advancements in the field, relevant to clinicians, researchers, healthcare professionals, regulators, nurses, patients, payers, pharmaceutical representatives, and all stakeholders in hematology.
Having introduced a new abstract category and scientific content at the EHA2024 Hybrid Congress, which focused on novel techniques, technologies, and analytical methodologies, this report underscores the significance of emerging technologies in hematology. These includes the empowering techniques of Artificial Intelligence (AI) and Machine Learning (ML), which are already driving advancements in clinical practice and patient care.
Whether you attended the Annual Congress or not, and whether you are an EHA member or not, this is an important overview that you cannot afford to miss!

Brian Huntly
EHA2024 Scientific Program Committee Chair

Disclaimer

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Gene Editing

Approaches to gene editing in β-hemoglobinopathies

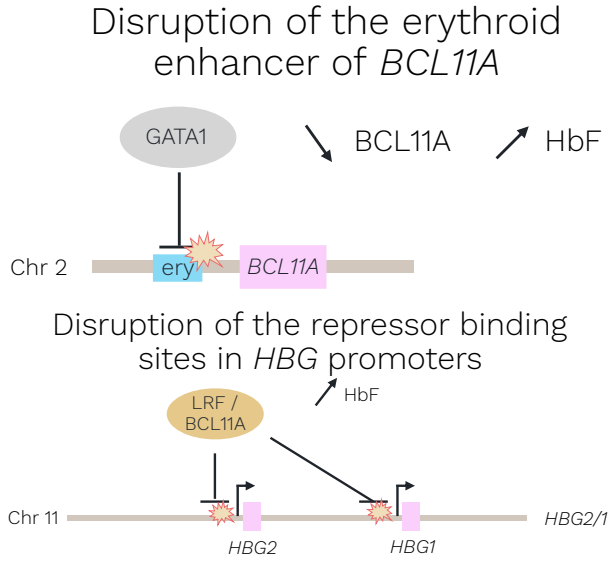
At the EHA2024 Hybrid Congress, Prof. Locatelli and Dr. Hardouin provided detailed introductions to gene editing and base editing, discussing the status of these technologies.[1,2]

Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are β-hemoglobinopathies with severe and potentially life-threatening manifestations.[3] Frequent blood transfusions in patients with SCD and TDT lead to several complications, primarily due to iron overload, despite the use of iron-chelating therapies.[4] Until recently, the only curative approach for SCD and TDT was allogeneic hematopoietic stem cell transplantation (HSCT).[5,6] However, only a minority of patients have an HLA-matched donor and this approach has limited success in patients above the age of 14.[1] As such, gene editing represents a promising alternative approach.

The two main strategies for gene editing in β-hemoglobinopathies are gene correction of the pathogenic hemoglobin beta (HBB) allele or reactivation of fetal hemoglobin (HbF) by reversing its repression either by disruption of the erythroid enhancer region of *BCL11A* or editing of the *BCL11A* binding motif in the promoter of hemoglobin 1/2 (*HBG1/2*).[7,8] Naturally occurring genetic variants cause a condition known as hereditary persistence of fetal hemoglobin (HPFH). These variants lead to reduced symptoms in patients with SCD and TDT.[9,10] Some gene editing strategies aim to mimic these variants in patients with β-hemoglobinopathies.

Two established approaches to gene editing in β-hemoglobinopathies are gene editing or gene disruption using CRISPR/Cas9 and base editing

Gene editing strategies for reactivation of HbF



using inactive or dead Cas9 (dCas9) or nickase Cas9 (nCas9) fused to a deaminase. In gene editing, CRISPR/Cas9 targets a specific sequence within the genome and creates a double-strand break (DSB). The DSB can be repaired by two different pathways, non-homologous end joining (NHEJ), which results in insertion/deletion (InDel) formation and gene inactivation, or homologous directed repair (HDR) where a donor template is provided, resulting in gene correction. However, this approach is not without its limitations. HDR is inefficient in quiescent cells and competes with NHEJ. Furthermore, CRISPR-mediated DSBs can activate DNA damage repair pathways ultimately leading to apoptosis, as well as genomic rearrangements. To overcome these limitations, base editing offers a promising alternate approach. Base editing uses inactivated Cas9, which retains the ability to target a specific sequence within the genome but doesn't generate a DSB at the target locus. The inactivated Cas9 is

fused to a deaminase. This system is advantageous because it doesn't result in DSB-induced toxicity, there is no InDel formation, and it is more efficient in quiescent cells.[11] Therefore, it is likely that base conversions in hematopoietic stem cells (HSCs) will lead to safer and more potent strategies.

Overview of selected pre-clinical studies and clinical trials of gene editing and base editing

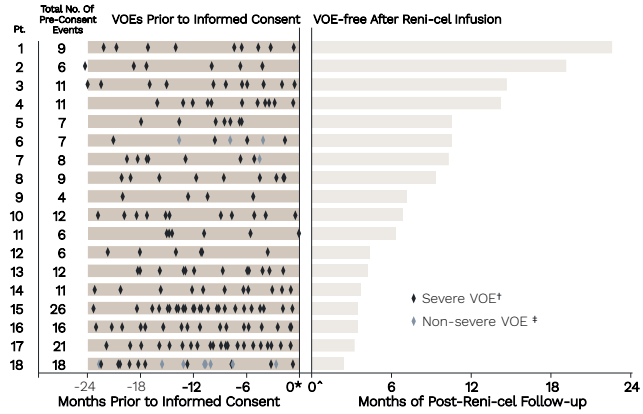
Gene editing

Several gene editing clinical trials have shown promise for curative therapies in β-hemoglobinopathies. The safety and efficacy of exagamglogene autotemcel (exa-cel) for TDT and SCD were assessed in two ongoing clinical Phase 3 trials, CLIMB-Thal-111 (NCT03655678) and CLIMB-121 (NCT03745287), conducted by Vertex Pharmaceuticals.[12,13] Exa-cel is a nonviral cell therapy designed to reactivate HbF synthesis through *ex vivo* CRISPR/Cas9 gene editing of the erythroid-specific enhancer of *BCL11A* in autologous CD34+ hematopoietic stem and progenitor cells (HSPCs).[12] In patients with TDT, treatment with exa-cel resulted in transfusion independence in 91% of patients[12], while in patients with SCD, it eliminated vaso-occlusive events (VOEs) in 97% of patients for 12 months or more.[13] The safety profile of exa-cel is consistent with myeloproliferative busulfan conditioning and autologous HSCT.[12] Exa-cel is approved by both the EMA and FDA for TDT and SCD.

CADPT03A12101 (NCT04443907) is a Phase 1/2 trial to assess CRISPR-Cas9-mediated disruption of the *HBG1* and *HBG2* gene promoters to induce HbF in patients with severe SCD, conducted by Novartis.[14] Another Phase 1/2 trial (NCT04211480), conducted by Bioray Laboratories, is evaluating CRISPR-Cas9-mediated disruption of the GATA1-binding site at the +58 *BCL11A* erythroid enhancer to induce HbF expression in children with TDT.[15]

The preliminary results of the Ruby Trial (NCT04853576), a Phase 1/2/3 study assessing the safety and efficacy of Reni-cel, were presented at EHA2024.[16] Reni-cel mimics naturally occurring variants of HPFH with edits to the *HBG1* and *HBG2* promoter regions to reactivate γ-globin expression and increase HbF production. The system utilizes proprietary AsCas12a instead of Cas9 to minimize off-target effects. Patients have been VOE-free for up to 22.8 months since Reni-cel infusion and experienced rapid correction of anemia, with sustained normalization of total hemoglobin (Hb). Increases in HbF and the percentage of F-cells were sustained at >40% and >90%, respectively. Patients also showed a trend in improvement or normalization of markers of hemolysis, including reticulocyte count, indirect bilirubin, LDH, and haptoglobin. No serious treatment-emergent adverse events (TEAEs) were reported as related to Reni-cel and the safety profile is consistent with myeloablative busulfan conditioning and autologous HSCT.[16]

VOE after Reni-cel infusion



Left panel ends at the informed consent date: 0* is day of informed consent. Right panel starts at the infusion date: 0^ is day Reni-cel was infused.
†A severe VOE requiring medical attention (despite hydroxyurea or other supportive care measures in the pre-treatment period) is defined as: an acute episode of pain with no cause other than a vaso-occlusion, resulting in either a ≥24-h hospital or Emergency Room (ER) observation unit or ≥2 visits to a day unit or ER over 72 h with both visits requiring administration of pain medications; acute priapism lasting >2 h and requiring a visit to a medical facility (with or without hospitalization); acute chest syndrome (ACS), which is defined as chest-wall pain in association with findings of a new pulmonary infiltrate on chest X-ray films associated with fever and/or respiratory symptom; or hepatic or splenic sequestration, which is defined as a sudden increase in organ size associated with pain in the area of the organ, decrease in the hemoglobin concentration of ≥2 g/dL within a 24-h period, and, for liver sequestration, abnormal change in liver function tests, including conjugated bilirubin, not due to biliary tract disease. ‡Non-Severe VOE is defined as an acute episode of pain with no medically determined cause other than a vaso-occlusion.

Base editing

Base editing is a relatively new technology, and while several proof-of-concept pre-clinical studies have been conducted, only one has reached the clinical stage to date.

For example, adenine base editing was used for gene correction of known β-thalassemia mutations in two proof-of-concept studies. In the first study, the HbE codon 26 mutation was converted to either wildtype (WT) or a normal variant hemoglobin (E26G) known as Hb Aubenas (asymptomatic trait phenotype).[9] The study showed high base editing efficiency and a significant increase in **β**-globin and Hb Aubenas mRNA. Importantly, base editing was maintained in repopulating HSCs. In a second study, the severe IS1-110-(G>A) β-thalassemia mutation was corrected.[17] Similarly, a high base-editing efficiency was observed, and the approach is safe as shown by transcriptome and mutation burden analysis.

In another pre-clinical study, an adenine base editor was used to reproduce the T>C HPHF point mutation known to create a Krueppel-like factor 1

(KLF1) activator binding site and a cytosine base editor was used to reproduce the C > T HPFH point mutations known to disrupt the LRF repressor binding site.[18] Not only does base editing allow for precise mutation insertion, but editing can also be extended to the inclusion of activator sites. In this study, base editing and recruitment of the KLF activator resulted in the more potent reactivation of HbF. An increase in F-cells was also observed. As expected, P53-related toxicity was relieved and fewer transcriptomic changes were seen compared to DSB-based approaches.

In the first base editing clinical trial, CS-101, a transformer base editor (tBE) is being investigated in 6 patients with TDT.[19] -114 is a naturally occurring single nucleotide variant (SNV) in the *HBG* promoter that leads to HPFH. Editing of the BCL11A binding motif in the promoter of *HBG1/2* by tBE triggers more robust HbF expression than editing of the *BCL11A* erythroid enhancer by CRISPR/Cas9. Through a dual-gRNA and specific inhibitor ("lock-key") system, tBE offers higher on-target editing efficiency and eliminates off-target mutations. To reduce off-target mutations while maintaining on-target editing efficiency, two single guide RNAs (sgRNAs) are used for colocalization at the target site. One sgRNA contains boxB hairpins to generate an R-loop region for intended base editing, and a helper sgRNA contains an MS2 hairpin to recruit APOBEC linked with a deaminase inhibitor. To transform the system to be reactive, a split-tobacco etch virus (TEV) system is employed. At the on-target site, the split TEV domains come into close proximity, forming a complete protease. This assembled TEV protease can access the TEV site and cleave the inhibitor, inducing efficient base editing.

The levels of mean HbF increased significantly from 8.1 to 129.1 g/L and the mean total hemoglobin increased from 101.2 to 129.5 g/L. At 3 months after infusion, the proportion of HbF-expressing red blood cells had reached 94% and then continued to rise and remained at ~98-99%. The safety profile is consistent with autologous HSCT, and at the data cut-off, there were no adverse events (AEs) or serious adverse events (SAEs) reported to be related to CS-101.

Design of the transformer base editor

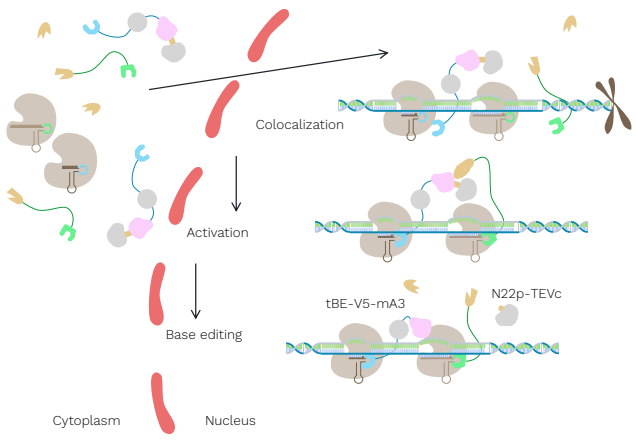


Image adapted from
Han W, et al. *Cell Stem Cell.* 2023:30:1624-1639.
Han W, et al. *Nat Protoc.* 2023;18:3194–3228.

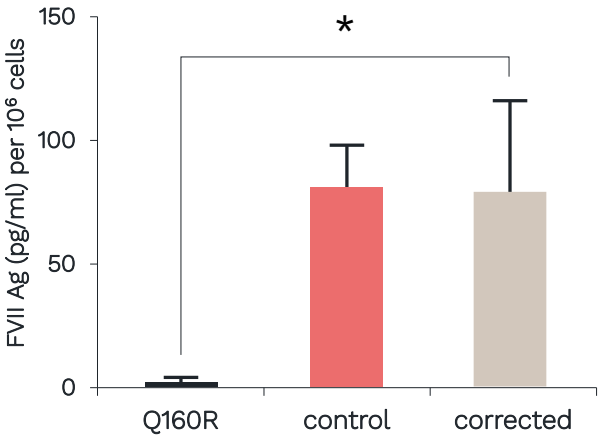
Outlook

Gene editing offers a potentially curative alternative to allogeneic stem cell transplantation, with safety data from trials aligning with the myeloablative busulfan conditioning and autologous HSCT. Base editing further advances gene editing by addressing several limitations, such as DSB-induced toxicity, reduced off-target effects, elimination of InDel formation, and increased efficiency in quiescent cells. The promising results from the first clinical trial of base editing in TDT presented at EHA2024 pave the way for future advancements and applications in the field. These potentially curative technologies hold the potential to revolutionize treatment approaches and improve patient outcomes significantly.

Autologous gene-corrected stem cell-derived hepatic organoids for the treatment of FVII deficiency

With the rapid advancement of both gene editing technology and pluripotent stem cells (PSCs) and their derived organoids, these tools are becoming increasingly valuable for various therapeutic applications. For example, as presented at EHA2024 by Dr Chollet, using a combination of disease modeling and disease correction, stem cell organoid technology together with gene editing can be used to correct common *F7* missense mutations in patient-derived induced PSCs (iPSCs).[20]

Profiling FVII antigen and activity in p.Q160R-HO



Patients carrying *F7* p.Q160R have low FVII activity (0,6-6,5%), low FVII antigen (10-28%) and variable bleeding phenotype.[21] Hepatic organoids derived from human iPSCs recapitulate liver biology, including the expression of coagulation factors. In this *ex vivo* preclinical study, the *F7* pQ160R mutation was successfully corrected in iPSCs of 3 patients with FVII deficiency.[20] The hepatic organoids were shown to express coagulation factors at similar levels to primary hepatocytes. The CRISPR/Cas9-mediated correction of the *F7* mutation enhanced FVII secretion and activity in the patient's hepatic organoids.

The development of a cell-based therapy for FVII deficiency holds significant therapeutic potential.

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02

Immune effector cell therapy

Next-generation CAR-T cell therapy and beyond

Introduction

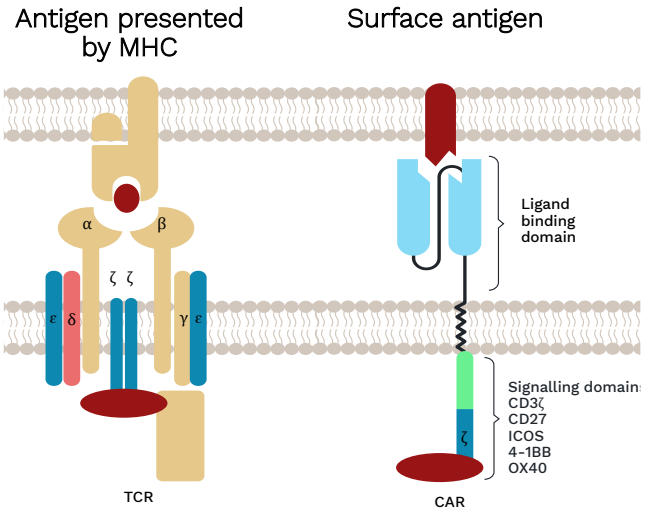
Immune effector cell therapy has become a staple of modern hematology. T-cells, engineered with chimeric antigen receptors (CAR), are the most commonly used type of immune effector cell employed in the fight against hematologic cancers. CARs, as opposed to natural T-cell receptors (TCRs), are single molecules containing an extracellular antigen-binding part and an intracellular T-cell activating part.

While the immune system in cancer patients produces endogenous T-cells that can recognize cancer cells and destroy them, they are not abundant enough and subject to central tolerance, as their targets are or resemble antigens that also occur in healthy cells. Adoptive T-cell transfer of engineered patient cells circumvents central tolerance, thereby making it possible to fight cancer cells effectively.

Despite great successes, most patients eventually experience relapse as the tumor develops different mechanisms of resistance. Current CAR-T cell therapies have a number of limitations, including:

- Toxic autoimmune effects, including Cytokine Release Syndrome (CRS) and Immune Effector Cell Associated Neurotoxicity (ICANS)[1]
- CAR-T cells are subject to local immunosuppression in the tumor microenvironment (TME)[2]
- CAR-T cells usually target only one surface antigen; antigen loss leads to resistance
- Conventional CAR-T cell therapy cannot be used against T-cell leukemia or lymphoma because they share the same surface antigens

Comparison of natural T-cell receptors and chimeric antigen receptors



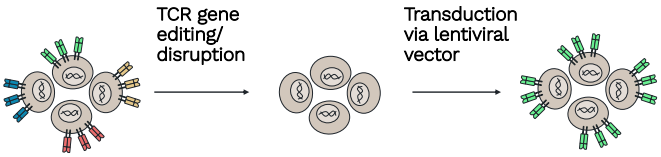
In the following section, we will present contributions from several speakers at EHA2024 who describe pathways toward solving these issues.

CRISPR-based gene disruption to engineer TCR-T cells against cancer

CARs are single molecules with antigen-binding domains from antibodies coupled with intracellular–domains that activate the TCR signaling cascade. Despite improvements in the choice of costimulatory molecules, insufficient CAR-T expansion and activation remain limiting factors for long-term suppression of tumor growth. Natural TCRs activate T-cell expansion

more efficiently, have a higher sensitivity for tumor antigens and have a lower risk for CRS and ICANS[1,3]. To create T-cells with natural TCRs against cancer, gene transfer alone is not sufficient, as the resulting engineered cells would have multiple competing TCRs. To solve this issue, the unwanted TCR can be knocked out via gene editing and a new TCR is introduced, e.g. via lentiviral transduction. Suitable TCRs can be identified in cancer patients. Cells harboring TCRs against tumor antigens can often be identified by their expression of exhaustion markers. T-cell receptor libraries against different antigen targets open the way for the use of natural TCRs in adoptive cell therapy. A clinical trial on the use of a WT1-targeting TCR-T cell is already underway[4]. Gene editing can also be used to increase T-cell fitness, prevent exhaustion and avoid suppression in the TME. While checkpoint inhibition is effective, it is also associated with a high risk of toxic side effects. Genome editing offers a way to change the intrinsic properties of T-cells, for example, via the knockdown of inhibitory receptors.

Creation of effector cells with natural TCRs



Prime editing: A new tool for advanced T-cell engineering

CRISPR prime editing is a weakened version of CRISPR, containing a nickase based on the Cas9 nuclease[5]. The nickase only creates single-strand breaks and does not require donor DNA. The technique enables all 12 base exchanges as well as small insertions and deletions via the prime editing guide RNA and reverse transcriptase. CRISPR prime editing is very precise and creates fewer off-target effects compared to conventional CRISPR-Cas9 gene editing. Despite its precision, prime editing bears the risk of off-target effects. To validate safety for patients, affinity for off-targets has to be analyzed for each prime editing module. OligoNucleotide Enrichment and sequencing (ONE-seq) is a screening method that uses synthetic DNA strands based on computational identification of closely matching sites, followed by in-vitro testing of the gene editor[6]. Since natural genetic variations can vastly change the risk for off-target effects between individuals, synthetic DNA with common variants is included in the analysis.

Next-generation CAR-T in myeloma

The most common reason for relapse after CAR-T therapy is antigen escape and antigen downregulation. Targeting additional antigens offers a solution to this problem[7]. The surface receptor GPRC5D is a promising alternative target in addition to BCMA, as it is highly expressed in plasma cells, including multiple myeloma (MM) but not in other immune cells or vital organs. CC-95266-MM-001 is a first-in-human phase 1 clinical trial in individuals with and without prior anti-BCMA therapy. At a dose of 1.5 x 10^8 cells, 40% of patients without and 60% of patients with prior BCMA treatment achieved complete response (CR). However, relapses are expected to occur in most patients, as advanced tumors are highly heterogeneous and contain cells with many different resistances even before therapy is initiated. A simultaneous attack on BCMA and GPRC5D could offer a way to target cells that lack either of the two antigens. Possible options include the simultaneous use of two CAR-T cell lines or cells expressing CARs against both targets. Experiments in mice, however, indicate that single-stalk CARs with double specificity are probably the most effective.[8]

A different approach is to treat patients at an earlier stage of progression before intrinsic resistance and a suppressive TME can form[7]. The clinical trial CAR-PRISM (NCT05767359) evaluates Ciltacaptagen Autoleucel in patients with smoldering MM.

By identifying and isolating T-cells with especially robust TCR signaling, CAR-T products can be optimized for longevity in the patient. The fitness of T-cells and their capacity to form memory cells can be further increased by accelerating the manufacturing process.[7,9] The culmination of this development is the production of CAR-T cells in patients, so-called direct-to-patient CAR-T cell manufacturing.[10]

WU-CART-007, an allogeneic CAR-T cell therapy targeting CD7 in R/R T-ALL/LBL

CAR-T therapy is widely used against B-cell malignancies, but its application in T-cell malignancies faces some inherent difficulties. Autologous CAR-T cells cannot be obtained from patients with T-cell malignancies due to the risk of contamination with malignant T-cells, which stems from malignant and healthy T-cells largely sharing the same antigens. Allogenic T-cells circumvent this risk but bear a high risk for Graft-vs-host disease (GvHD). Furthermore, T-cell antigen-targeting CAR-T cells attack each other by default. The allogenic CD7-targeting WU-CART-007 CAR-T product is designed to circumvent all of these problems[11]. Knockout of T-cell receptor alpha constant (TRAC) reduces the risk of GvHD, while deletion of CD7 avoids the risk of “friendly fire”. The product is being tested in the WU-CART-007 1001 clinical trial (NCT04984356) in patients with refractory or recurrent (R/R) acute lymphocytic leukemia (ALL) and R/R lymphoblastic lymphoma (LBL). The therapy was

effective in this heavily pretreated patient collective, achieving 100% overall response rate (ORR) and 67% complete response rate (CR) or CR with incomplete count recovery (CRi) at the recommended phase 2 dose (RP2D). Safety was considered manageable, with CRS being the most common Gr≥3 TRAE, affecting 31% of patients at RP2D. Two Gr4 cases of CRS occurred but were manageable with supportive care.

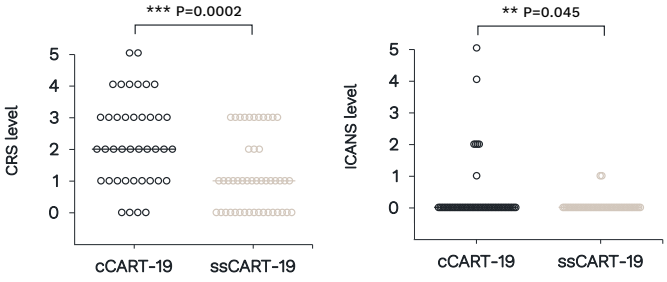
Armored huCART19-IL18 in patients with R/R lymphomas who progressed after anti-CD19 CAR T-cell therapy

Aggressive tumors evolve a suppressive TME that protects cancer cells from interventions of the immune system. The mechanisms that suppress endogenous immune cells also affect CAR-T cell activity. Altering the cytokine signals CAR-T cells receive can increase their fitness and restore tumor cell-killing ability. The clinical trial NCT04684563 evaluates huCART19-IL18, a CD-19-targeting CAR T-cell product with the capacity to secrete transgenic IL-18, in patients with R/R lymphomas who progressed after anti-CD19 CAR-T cell therapy[12]. IL-18 enhances the cytolytic potential, modulates the TME and has anti-lymphoma effects. ORR was 81%, while CR was 52%. At a median follow-up of 17.5 months, the median duration of response (mDOR) was determined at 9.6 months. 62% and 14% of patients experienced CRS and ICANS, respectively. Only 14% of patients had CRS Gr3; all cases of ICANS were Gr1 or 2. Other common AEs included neutropenia (68%), general leukopenia (62%) and decreased platelet count (51%). In summary, the treatment with armored huCART19-IL18 is feasible and did not raise unexpected safety concerns. huCART19-IL18 results in durable responses even after the prior failure of anti-CD19 CAR-T therapy.

High efficacy and safety of IL-6-knockdown CD19-targeted CAR-T cells in R/R B-ALL patients

While signaling from some proinflammatory cytokines can enhance tumor cell killing, it can also lead to CRS and ICANS, which are experienced by most patients who receive CAR-T products. IL-6 is considered to be one of the key factors responsible for those adverse effects. ssCART-19 is a new CAR-T cell product simultaneously expressing CAR structures and an *IL-6* silencing element, thereby controlling the overall release intensity of cytokines[13]. The shRNA insertion was confirmed to not affect CAR-T amplification. It was hypothesized that ssCAR-T-19 could mitigate severe CRS while preserving the available anti-tumor effect in patients with R/R B-ALL. This hypothesis was tested in a clinical trial (NCT04825496) on R/R B-ALL patients.

CD19-loaded EVs are highly specific for CD19-targeting CAR-T cells



Indeed, the rates and severities of CRS and ICANS were significantly lower in the ssCART-19 group compared to the classical CART-19 (cCART-19) group. Peak levels of IL-6, IL-2 and TNFα were significantly lower in the ssCART-19 group compared to the cCART-19 group. Response rates, progression-free survival and overall survival were not significantly different between groups, with a (non-significant) tendency to better survival with ssCART-19.

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03

Multi-omics

The advent of high throughput multi-omics technologies

The term 'omics' encompasses the extensive exploration and analysis of data that depict the structure and function of biological systems across various levels. Omics technologies have significantly contributed (and continue to contribute) to a better understanding of hematological malignancies, which can be highly heterogeneous, with many variable subtypes and different molecular profiles. The constantly expanding array of omics technologies and their capacity to yield extensive information necessitate the recognition that no single technology can comprehensively fulfill all needs. Consequently, it is imperative to perceive omics technologies as supplementary, as 'Multi-omics,' each possessing its strengths and limitations. Different levels of information (spatial, cellular, molecular, etc.) can be obtained with multi-omics technologies that provide a clearer picture of the complex mechanisms underlying hematological malignancies. For instance, proteomic technologies can see defined states of cell populations, while genomic and transcriptomic technologies allow for finer classifications.

Combining different omics for better management of hematological malignancies

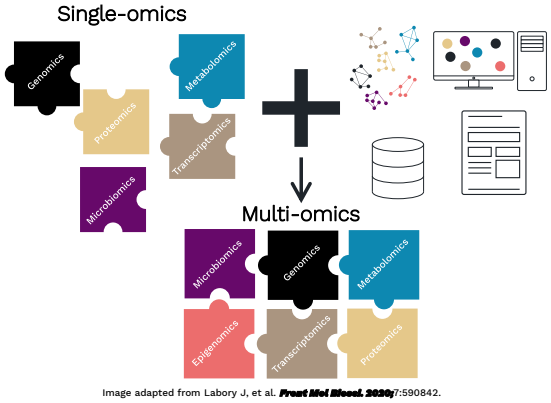


Image adapted from Labory J, et al. *Front Mol Biosci.* 2020;7:590842.

Multi-omics-based approaches are starting to make their way into diagnosis, disease monitoring, and transformational approaches to discover new therapies. They could play a pivotal role in clinical decision-making and lead to more personalized patient care.

Latest developments in single-cell multi-omics technologies

Several single-cell multi-omics technologies, which can be used for the study of hematological malignancies, were presented at EHA2024.

Botta and colleagues developed FlowCT, a computational approach that allows semi-automated analysis of large flow cytometry data sets, including automated clustering and further statistical analyses.[1,2]

Other recently developed technologies also take advantage of traditional flow cytometry methods. Using traditional flow cytometry staining protocols, CITE-seq and Ab-seq analyses provide transcriptomic and proteomic information from individual cells. These technologies are multimodal approaches based on the use of oligonucleotide-conjugated antibodies for simultaneously profiling single-cell mRNA and surface proteins.[3,4]

MAESTRO is a computational workflow for the integrative analysis of gene expression and chromatin accessibility at single-cell resolution using scRNA-seq and scATAC-seq, which can help identify transcriptional regulators of malignancy in specific cell populations.[5]

A new single-cell technology called Zman-seq allows for the real-time recording of transcriptomic dynamics. This technology was presented in the context of studying how the tumor microenvironment changes across time in response to treatment with antagonistic anti-TREM2 molecules. The use of Zman-seq may help us better understand how the immune system adapts to treatment and could lead to the

development of more effective immunotherapies.[6,7]
Finally, a highly multiplexed imaging method called IBEX was presented. It uses fluorescently labeled antibodies for spatial proteomic profiling of tissues. This versatile technology can process over 60 antibodies in one sample and produce high single-cell and spatial resolution images, which can help study rare cell populations.[8,9]

Clinical applications of single-cell multi-omics technologies

Radtke et al. used a combination of sequencing and imaging technologies to profile RNA and proteins in excisional biopsies from follicular lymphoma (FL) patients enrolled in a prospective clinical trial, and key spatial findings were then validated in a larger cohort using IBEX technology. This study created a comprehensive multi-omic atlas of the FL tumor microenvironment that can help predict disease outcomes. Unique follicular growth patterns were observed 20 months before relapse, and increased stromal remodeling and extracellular matrix deposition were seen in more aggressive clinical cases.[8,10]

Using single-cell genomic analysis in pre-treatment peripheral blood (PB) samples from diffuse large B-cell lymphoma (DLBCL) patients who received CAR T therapy, distinct myeloid compositions and molecular states were found to effectively predict responses to CAR T treatment. Specifically, a strong downregulation of myeloid cells as well as an enrichment of malignant and healthy-like B-cells, characterized by an unbalance in κ/λ light chain gene products and CNVs in regions associated with B-cell malignancies (e.g.: *BCL2*), were associated with favorable responses to CAR T therapy.[6]

The single-cell MRD (scMRD) assay is a multi-omics approach that integrates genotypic and immunophenotypic profiling in individual bone marrow cells and allows MRD detection with high sensitivity and specificity. scMRD assessment of samples from 2 AML patients who were MRD-negative by flow cytometry detected AML clones and revealed a highly complex clonal architecture, including clone-specific acquired mutations, zygosity, genome-wide structural variations, and surface immunophenotypes.[11] Even though this technology may not be applicable in routine clinical practice yet, the researchers believe that understanding the biology that allows MRD clones to resist therapy is necessary to guide the development of more effective and personalized treatments.

Phosphoproteomic technologies for precision hemato-oncology

By measuring phosphorylation sites and, thus, kinase activity, phosphoproteomic tools can help us quantify oncogenic signaling in a way that considers many molecular events involved. Many studies have evaluated the correlation between the activation of kinase signaling pathways and responses to targeted therapies.[12]

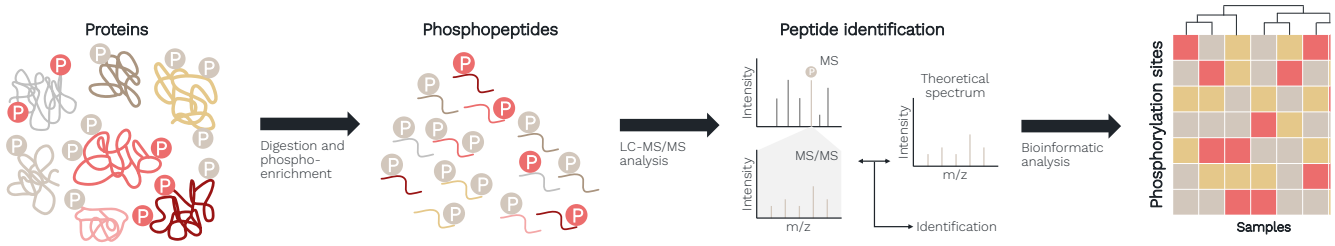
Results from a retrospective clinical study presented at EHA2024 demonstrate that phosphoproteomic analysis can predict response to midostaurin plus chemotherapy (M+IC) in acute myeloid leukemia (AML).[12] Previously, genetic mutations alone failed to predict the best treatment for a given patient, as illustrated by the fact that >40% of *FLT3*^{mut} patients in the RATIFY phase 3 trial were refractory to midostaurin.[13] A phosphoproteomic signature identified in a previous preclinical study[14] was validated in 47 *FLT3*^{mut} AML patients treated with M+IC, with better responses observed in patients positive for the kinase activity signature.[12]
The future scope would be to move to a patient-centric approach using machine learning (ML) on phosphoproteomics data to rank treatments based on predicted efficacy within each patient.

Enhancing prognostic value in DLBCL by incorporating radiomics models

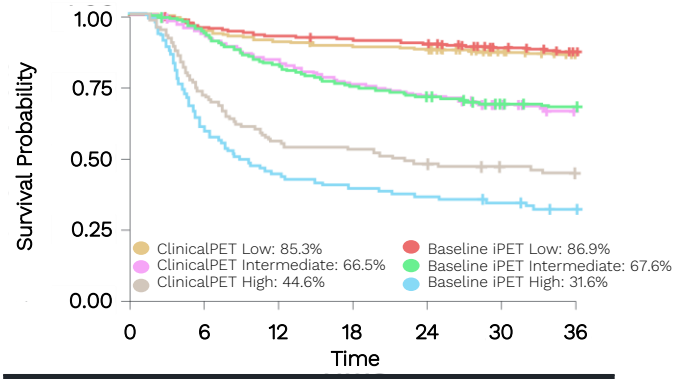
Radiomic features, which can be extracted and calculated from PET scans, provide a comprehensive quantification of tumor phenotype and may help predict disease outcomes. Several software available for high-throughput extraction and calculation of radiomics features can extract up to 500 different features, including morphological, intensity, and texture features.[15]

Among radiomics models currently used in DLBCL, International Metabolic Prognostic Index (IMPI) is the best known. It uses metabolic tumor volume (MTV) as a continuous variable in addition to the age and stage of the tumor.[16] ClinicalPET, on the other hand, incorporates MTV, SUV_{peak}, and maximum distance between the target and another lesion ($Dmax_{bulk}$). The ClinicalPET model can identify high-risk patients better than the IMPI

Phosphoproteomics workflow



Survival curves of DLBCL patients according to the ClinicalPET and baseline iPET models



ClinicalPET Number at risk							
Low	608	574	547	537	531	504	486
Intermediate	304	282	253	228	216	201	192
High	102	72	56	53	48	44	42

Baseline iPET Number at risk							
Low	608	582	563	553	545	518	498
Intermediate	304	285	249	226	214	198	193
High	102	61	44	39	36	33	29

model. The baseline iPET model, where interim PET response was added to the baseline radiomic features, showed significantly improved performance in predicting survival rates of high-risk patients in DLBCL (44.6% with ClinicalPET vs 31.6% with baseline iPET).[17]

Simple radiomics models are easy to use, but more complex models require harmonization before they can be used in a multicenter setting. AI-based methods for automatically segmenting lesions and making direct predictions from PET scans could help integrate radiomics into routine clinical practice.

With the advancement of various multi-omics technologies, their ability to effectively predict disease outcomes and treatment responses in hematological malignancies has been demonstrated. However, integrating these technologies into regular clinical practice is currently a challenge due to their high costs and the complexity of data analysis. Machine learning (ML) and artificial intelligence (AI)-based tools could potentially simplify the data analysis and interpretation of complex results obtained from these promising technologies.

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04

AI and machine learning

AI-based tools and applications in hematology

The AI revolution and its impact in the field of hematology

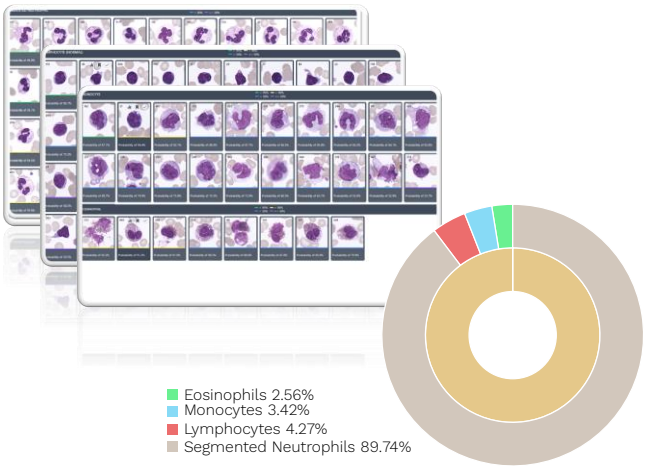
Ever since ChatGPT was launched, the field of artificial intelligence (AI) has made a profound impact on our society. AI promises to enhance productivity, boost efficiency, increase insights into big data and transform the life cycle of medicines, healthcare and public health. This year at the EHA Congress in Madrid, AI stood true to this promise, with several abstracts describing the innovative use of AI and machine learning (ML) presented, the summary of which will be covered in this section. But before diving into them, let's clarify the difference between them. While AI and ML are often used interchangeably, ML is a subset of the broader category of AI. ML works to computationally extract and predict significant outcomes from intricate data structures through learning from training data, while AI is a system that autonomously reasons, comprehends, and acts to solve complex problems replicating human intelligence.[1]

The impact of AI is anticipated to be substantial in various aspects of hematology practice, and the potential areas to enhance patient care using AI in hematology include enhancing patient-physician interaction, automating diagnosis and screening, personalizing treatment phase, monitoring patient health and engagement and supporting in research and clinical trials.[2,3]

AI in automated diagnosis

The assessment of bone marrow morphology is crucial for diagnosing hematological malignancies. However, these assessments are often labor-intensive, time-consuming, and not entirely reproducible. ML can process medical image data for diagnostic purposes and is becoming increasingly superior to human examiners in accuracy and speed. A prospective, blinded clinical trial (BELUGA, NCT04466059) compared an AI-driven cloud-based platform to conventional manual examination in peripheral blood differential count with >29,000 cases. The results showed a 94.5% concordance between the two methods, with the AI-driven platform demonstrating higher reproducibility and shorter turnaround times than the manual approach.[4,5]

An AI-based approach for classifying blood cell subtypes from peripheral blood smears using single-cell images



By integrating cytomorphological features using ML, a computational method Haemorasis, was able to distinguish a specific mutant form of MDS (*SF3B1*) from other myelodysplastic syndrome (MDS) forms using only cytomorphology and blood counts, with high predictive performance.[6] Another example was a multi-step deep ML approach that automatically segmented cells from bone marrow images, accurately distinguishing between acute myeloid leukemia (AML) samples and healthy controls, and also predicted the common AML mutation *NPM1* using only image data.[7]

SCEMILA, a single-cell-based explainable AI model, was developed for the classification of AML subtypes from blood smears based on over 80,000 single WBC images from 129 AML patients and 60 healthy controls. SCEMILA could perfectly discriminate between AML patients and healthy controls, predict the AML genetic subtypes with high accuracy and identify clinically relevant cells.[8] And finally, a deep learning algorithm for detecting *MYC* rearrangement in scanned histological slides of diffuse large B-cell lymphoma (DLBCL) enabled a simple and fast prescreening, leading to an approximately 34% reduction in genetic tests.[9]

Collectively, these works revealed the potential to develop a unifying, dynamic model, almost a virtual guide, that integrated different data sources to find the best possible diagnosis for each patient with limited human interference.

AI in screening

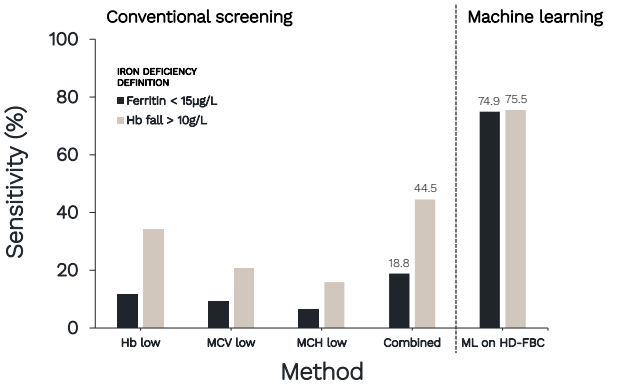
Conventional screening tests using full blood count may fail to identify iron deficiency (ID) in many patients, especially medically vulnerable patient groups. High-dimensional full blood count (HD-FBC) often includes a wealth of summary statistics and information that are typically overlooked. A 2-part study was presented to transform ID screening using ML to detect ID at lower costs and with higher sensitivity than current screening. Data from 48,000 blood donors from the INTERVAL trial was used for the analysis.[10]

In part 1 of the analysis, the sensitivity of current ID screening on a larger scale using two ID definitions was investigated.[11] All single low-thresholds for Hb, MCV, and MCH showed low sensitivity and combining them improved sensitivity but still remained under 50%, with over half of the ID cases being missed. Sensitivity for detecting ferritin <15 µg/L using national full blood count (FBC) reference ranges within a healthy population was also low. Part 2 involved training an ML model (XGBoost) using 250,000 outpatients from Cambridge University Hospitals previously tested for ID to detect ferritin <15 µg/L from HD-FBC data. The proposed model on the HD-FBC reached higher sensitivities of about 75% for both definitions of ID. These results indicate that an ML algorithm based on routine FBC test results can accurately predict low ferritin levels in anemic patients. Moreover, implementing the computational algorithm in the laboratory test result system could assist physicians and specialists in laboratory medicine, thereby reducing the number of unidentified IDs. The ML model was validated in a second cohort with an ethnically diverse donor population, aimed at enhancing the sensitivity of models using neural networks. A similar analysis had been performed in already secured patient data of approximately 2.4 million patients at partner institutions.

AI in clinical trials

The collection of patient data to generate clinical evidence in the field of hematology can be quite challenging, particularly when dealing with rare diseases. Moreover, privacy concerns might limit the utilization of data to certain contexts.[12]

Comparing conventional and machine learning screening models for the detection of iron deficiency using two definitions



Synthetic data can help overcome many of the above-mentioned drawbacks of working with real patient data, enabling faster, less expensive, and more scalable access to information that is representative of the original source while preserving privacy. Synthetic data consists of AI-based generative models characterized by multi-layer neural networks that can generate samples by learning the distribution of a set of real patient data. Generative Adversarial Networks (GANs), a deep learning-based method, can generate artificial outputs, which are then passed to the discriminator along with real data to identify which outputs are real and which are fake. A conditional GAN that ensures more precise generation and discrimination modeling of large data sets with complex distribution and interactions among different features was presented.[13] A synthetic validation framework (SVF) was also developed to evaluate the fidelity and privacy preservability of the newly generated synthetic data. A cohort of real MDS patients from GenoMed4All was used to generate and validate the synthetic data in different experimental settings. The optimized conditional GAN method was able to recapitulate the clinical and genomic properties of real patients with myeloid neoplasms, which are rare diseases characterized by large clinical and biological heterogeneity. The distribution of data points was similar between real and synthetic cases, including mutation frequency and survival. In the future, synthetic patient datasets could be used to improve clinical trials by reducing costs and ensuring all participants receive active treatment, for e.g., Alectinib, Avelumab, and Blinatumomab were approved based on studies that included comparator arms with synthetic patient data or patient records.[14] This technology may increase the scientific use and value of real data and

accelerate precision medicine in hematology, ensuring high privacy preservation of newly generated synthetic data.

AI in research

Flow cytometry offers a high-speed quantitative multiparameter analysis of cells in suspension, whereas microscopy offers a more versatile option to examine the cell-to-cell interactions, cell morphology and other parameters (e.g., Dutcher bodies). Attune™ CytPix™ flow cytometer combines an acoustic flow cytometer, which is ten times faster than a conventional flow cytometer, with a high-speed bright-field camera with multiple configurations possible.[15] With a modular design for 2 to 4 laser systems, up to 14-color flow cytometry and a violet 6-channel configuration, it is able to capture up to 6000 images per second with the ability to select which population to image and size measurement tool using images. The AI-powered CytPix flow cytometer software has the ability to automatically analyze bright-field images with and without fluorescent labels to improve gating strategies and identify rare cells. This versatile and powerful technology is currently used for research purposes only to study rare cell populations, label-sensitive cells, apoptosis, DNA content and Ki-67 expression.

The use of another innovative AI tool was presented by researchers from the University of Perugia.[16] Since the launch of AlphaFold, the use of deep learning AI systems to predict the 3D structures of protein using only its one-dimensional amino acid sequence has accelerated scientific research and discovery globally.[17] In a study presented by Martarelli et al., AlphaFold2 predicted the 3D structure of different anti-CD30 mAbs for the development of CD30 CAR T-cells against Hodgkin's lymphoma. The selection of mAb-derived single chain fragment variable (scFv) is a crucial step in CAR construction to ensure accurate and effective signaling upon tumor antigen binding. Current scFv screening methods are expensive and time-consuming, so developing faster and more cost-effective methods is extremely important. Molecular Dynamics (MD) simulations were performed to identify the most stable complex and calculate the energy required to dissociate the antibody-antigen binding.

Two of three anti-CD30 mAbs were newly generated (clone 142 and clone 231), while the other one was already commercially validated (clone BER-H2). In silico molecular docking analysis showed that clone 142 mAb exhibited the highest affinity for CD30, which was further confirmed by surface plasmon resonance. Clone 142-derived CD30 CAR T-cells also displayed higher cytotoxicity in vitro as well as a higher cell proliferation activity and pro-inflammatory cytokine release compared to other antibody clones-derived CD30 CAR T-cells. In vivo experiments also showed that clone 142-derived CD30 CAR T-cells effectively eradicated HD-LM2 cells and remained in remission even after tumor rechallenge. These results highlighted the potential to streamline the selection of scFv from mAbs and advance the development of CAR constructs. The use of AI systems could substantially reduce time, costs, and the need for laboratory animal use. The next areas of application for these AI-guided, in silico analyses, could involve the identification of the optimal scFv orientation, the finest linker, and the ideal space length.

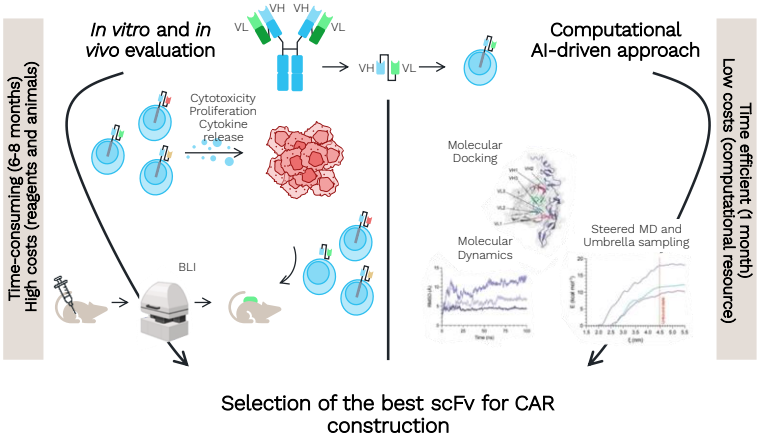
AI in personalizing treatment

The presence of intratumoral heterogeneity and the evolution of multiple cancer subclones are major reasons for resistance to treatment in advanced hematological malignancies. This necessitates personalized and possibly combination therapies to enhance patient outcomes. However, identifying patient-specific treatments is difficult due to the large number of possible drug-dose combinations and limited patient cells for testing. [18]

The major flaws of current ML algorithms are that they do not target cancer subclones and lack preclinical dose-specific responses and toxicity predictions. An effective machine learning (ML) approach based on XGBoost was tested to predict the most synergistic drug-dose combinations in 4 AML patient samples by combining single-cell RNA sequencing with ex vivo single-agent drug testing in individual patient-derived primary cells. [19]

The approach accurately predicted patient-specific drug combinations resulting in synergistic co-inhibition and targeting specific AML cell subpopulations, but they could not be applied to patients whose tumors are not easily amenable to drug testing.

Difference between the classical and computational AI-driven approaches to selecting the best scFv for CAR construction



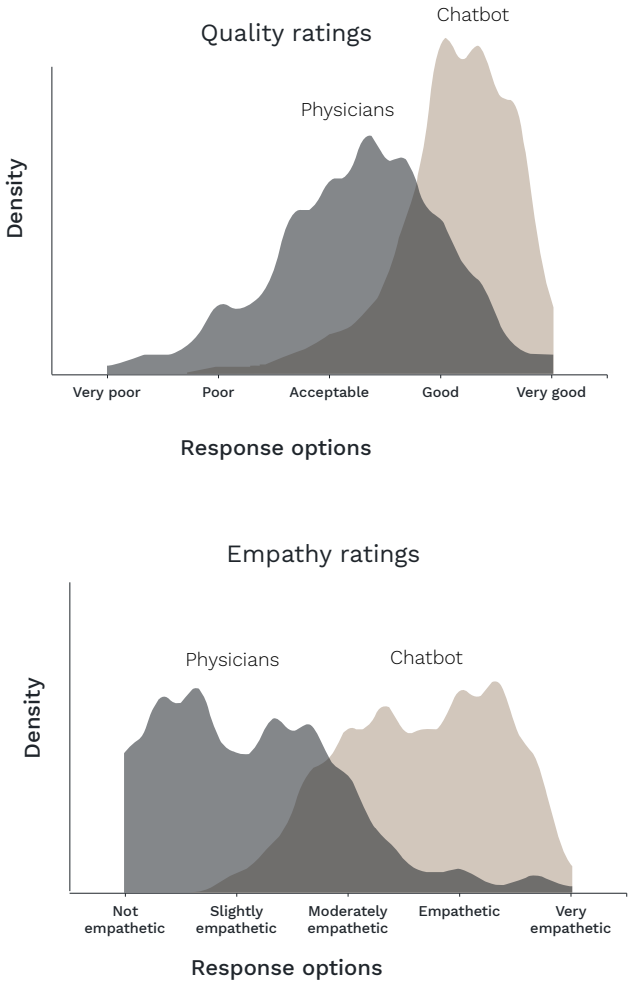
Selection of the best scFv for CAR construction

scTherapy, a machine learning model that uses scRNA-sequencing data to identify cancer-selective and low-toxic multi-targeting options for individual cancer patients, was presented.[18,20] Predictions were based on transcriptomic differences between genetically distinct cancer cell populations in individual patient samples. By pre-training a gradient boosting model (LightGBM) that leverages a massive reference database of large-scale phenotypic profiles measured in cancer cell lines in response to single-agent cell viability assays, a ranked list was generated of the most effective multi-targeting options for 4 AML patients. This made functional *ex vivo* drug testing more feasible by prioritizing the most potent, multi-targeting options for further experimental validation in scarce patient cells. The *ex vivo* drug testing found 96% of the multi-targeting treatments to display selective synergy, and 86% exhibited low toxicity to normal cells. Future studies were planned to tailor input data to be patient-specific and drug-class-specific by considering differences in binding affinities, phenotypic profiles, and treatment time points.

In conclusion, AI has an immediate impact and assists in the better and more personalized management of individual patients. The AI-based systems presented at EHA2024 accelerate clinical innovation by facilitating the design of more efficient clinical trials that require fewer patients, thus speeding up the availability of drugs. This is particularly advantageous for hematology, as it deals with rare diseases, which are quite heterogeneous in their presentation, and the diagnosis and recruitment of these patients in clinical trials can be time-consuming. It also raises the question of whether AI-based systems will support or potentially replace the traditional doctor-patient relationship. In a recent study, a chatbot provided high-quality and empathetic responses to patient questions in an online forum.[21]

The study highlighted the potential for utilizing AI assistants for messaging, an area that has been previously overlooked. Implementing AI-assisted communication could increase productivity, allowing clinical staff to focus on more complex tasks. Additionally, it may reduce unnecessary clinical visits, freeing up resources for those who truly need them. AI can help healthcare professionals perform their tasks more effectively and efficiently, ultimately improving patient care.

Density plots illustrating the disparity between responses from physicians and chatbots to patient questions



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05

New tools for diagnosis & testing

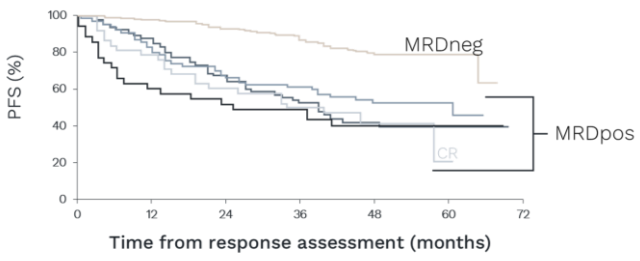
Introduction

The understanding of diseases on a molecular level moves forward quickly, enabling physicians to personalize therapy adjusted to genetic variants and risk factors. Furthermore, new tools to monitor diseases are on the horizon, delivering improved insights into the development and prognosis of diseases.

Recent and future tools for response assessment in multiple myeloma (MM)[1,2]

Clinical outcome trials in MM are based on the measurement of conventional response criteria, including overall survival (OS), progression-free survival (PFS) or overall response rate (ORR). However, new standard of care (SoC) drugs are nearing 100% ORR and significantly prolong survival outcomes.[3,4] Thus, ORR might no longer be the best endpoint. Minimal residual disease (MRD) status could reduce the time required to evaluate new treatments. Long-term data from the PETHEMA/ GEM2012MENOS65 study showed no differences in PFS and OS between all MRD positive (MRD+) groups, including patients with complete response (CR) but a clear difference to MRD negative (MRD-) patients.[5] Additionally, a meta-analysis showed that MRD- is strongly associated with better outcomes in all disease settings.[6]

Difference in the Progression-free survival (PFS) based on the MRD status[2]



MRD assessment in MM uses different methods, including next-generation flow (NGF), next-generation sequencing (NGS), and positron emission tomography/computed tomography (PET/CT).[7] Clinical trials should assess MRD in patients with at least very good partial response (≥VGPR) to ensure all patients achieving MRD- are captured.[8] MRD assessment is feasible in clinical trials and yields accurate and sensitive results. MRD-CR was therefore approved by the FDA as an early endpoint in clinical trials for accelerated drug approval.[9]

Methods for MRD assessment must fulfill the following requirements to achieve the status of a clinical standard:

- Standardized method
- High sample quality
- Analysis of sufficient amounts of cells
- Use of first bone marrow aspirate pull
- Systematic check for hemodilution
- Calculated the limit of detection (LOD) in each sample and defined MRD threshold
- Several time points for assessment, as kinetics are more informative

Regular bone marrow aspirations are highly invasive and even high-quality bone marrow samples might not be representative due to the patchy distribution of myeloma cells and extramedullary disease (EMD). Therefore, complementary methods, e.g. circulating tumor DNA (ctDNA) or light chain mass spectrometry, are used to reduce invasiveness, monitor MRD development and reveal EMD.[10]

The combined approach could help define new patient subgroups or response categories based on MRD sustainability. For example, blood mass spectrometry was used in the GEM CESAR trial with high-risk smoldering MM patients and was able to segregate two groups of patients in the CR group with different PFS outcomes.[11]

Reaching sustained MRD- is desirable but difficult to assess in routine practice since MRD status is dynamic and continuous monitoring is necessary. Underlining the importance of MRD kinetics, Guerrero and colleagues analyzed nearly 1,800 MRD assessments and were able to identify three unique patient subgroups:

- Sustained MRD: Excellent outcome
- Detectable but stable MRD: Mild detriment in PFS but not OS
- Detectable and evolving MRD: Reduced survival[12]

A combined scoring system based on different prognostic markers including MRD- could serve as a predictor for treatment cessation.[13,14] In addition to MRD assessment via sampling, big data approaches will gain importance in outcome prediction in MM. Novel machine learning models can predict MRD outcomes at diagnosis based on genetic, tumor, and immune biomarkers or help predict an MGUS-like phenotype using flow cytometry data.[15,16]

MRD assessment is an informative and powerful, yet not perfect biomarker in MM. It will not replace but rather support genetic risk assessment and allow, together with additional methods, a more personalized treatment decision-making.

Ultrasensitive biosensor for non-invasive diagnosis of T-ALL/LBL

T-cell acute lymphoblastic leukemia (T-ALL) and T-LBL (T-cell lymphoblastic lymphoma) are highly malignant neoplasia of T-cells and T-precursor cells, occurring mostly in children. They are rapidly progressing and have a poor prognosis.[17] Standard diagnostic tools are expensive, invasive and lacking in sensitivity. Liquid biopsy as an alternative is limited by low target content in samples, high costs, and low sensitivity and specificity. The CRISPR/Cas system has emerged as a new method that is highly efficient, target-specific and easy to handle and adapt, but lacks applications for multiplex detection and requires multiple reaction steps.[18] Wang et al. presented an affordable, fast, sensitive and specific CRISPR biosensor for one-step/one-pot diagnostics in T-ALL/T-LBL.[19]

Cas9 recognition specificity and enrichment for the target gene sequence were increased successfully and the system is ready for use in isothermal amplification. Using different Cas effectors and their trans cleavage ability together with reporter DNA/RNA labeled with different fluorophores allows multiple signal visualization.

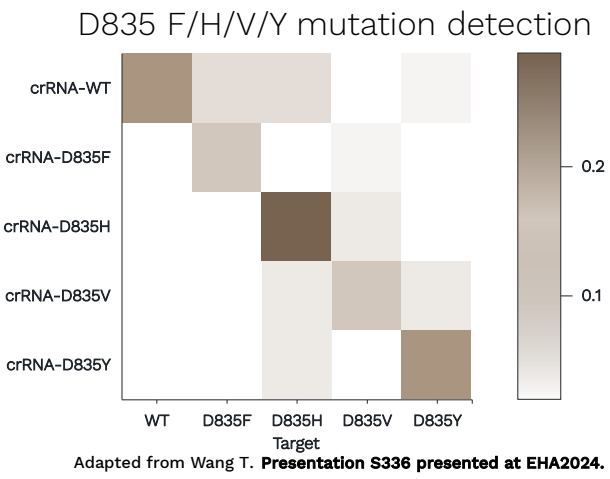
Optimizing all steps mentioned, the whole approach can be conducted more quickly and in one reaction tube throughout, delivering outcomes for several genetic biomarkers simultaneously.

A modified Cas9 endonuclease was engineered in which one endonuclease domain was deactivated. The new enzyme, referred to as Cas9n, is a nickase that only cleaves one of two DNA strands. Further improvements via structure-directed mutagenesis based on rational design yielded the "Hi-Fi Cas9n" enzyme with improved target specificity (off-target activity) and faster kinetics (off rate) while enhancing amplification efficiency. Cas9n is highly specific and can detect point mutations in the vicinity of PAM and the cleavage site.

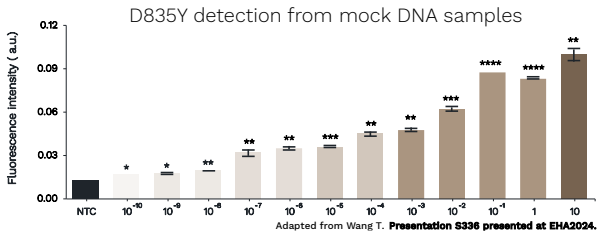
Another enzyme, the Cas13a effector, was improved by introducing synthetic mismatches in critical positions leading to increased and highly specific single-base mutation discrimination.

Specificity for SNVs in the *FLT3* gene was successfully tested by using different CRISPR-RNA (crRNA) samples on the wild-type *FLT3* gene and D835F/H/V/Y mutations.

Detection of specific SNVs in the *FLT3* gene using various CRISPR-RNA (crRNA) samples by an ultrasensitive biosensor



Adapted from Wang T. **Presentation S336 presented at EHA2024.**



Adapted from Wang T. **Presentation S336 presented at EHA2024.**

The system can detect multiple SNVs at extremely high sensitivity. Cas12a and Cas13a modules were able to consistently detect targets at 0.1 aM ($1x10^{-18}$M) concentration, which corresponds to an analytical limit of detection of 1.2 copies per reaction. This result is equivalent to PCR and superior to other CRISPR-based methods.

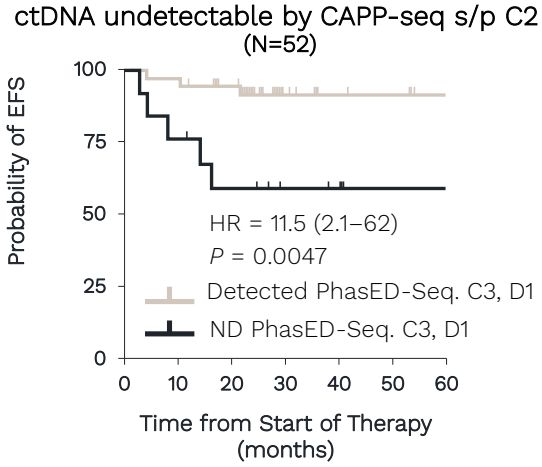
The technology's ability to perform multiplex analysis was confirmed with samples of known genes involved in T-ALL/T-LBL (*IDH, FLT3, KRAS*).[20,21] CRISPR-based diagnosis can already serve as a tool for SNP detection in T-ALL/T-LBL. However, with optimized components, a less time-consuming one-step/one-pot multiplex approach seems feasible. The presented method

is quick, highly specific, and sensitive, but further validation with patient samples is needed.

PhasED-seq for MRD detection

Conventional MRD testing involves the detection of circulating tumor (ct) DNA via single nucleotide variations, e.g. via CAPP-seq. PhasED-seq detects phased variants, which are multiple mutations occurring on the same cell-free DNA molecule. Phased variants are enriched in stereotyped genetic regions in lymphoma and are associated with the activity of certain enzymes, e.g. AID. Their detection significantly reduces background error rate, thereby increasing sensitivity. PhasED-seq lowers the detection limit for minimal residual disease (MRD) by a factor of up to 100, compared to CAPP-seq, which could help identify more patients with Diffuse Large B-cell Lymphoma (DLBCL) who are in need of further treatment.[22]

Kaplan-Meier plot showing the event-free survival (EFS) for 52 DLBCL patients who are ctDNA-negative by CAPP-Seq after 2 cycles



PhasED-seq was tested in DLBCL patients undergoing a treatment regimen of 6 cycles of chemotherapy. Of 52 patients who tested MRD- via CAPP-seq after Cycle 3, 13 (25%) tested positive via PhasED-seq. PhasED-seq was effective in delineating the risk of recurrence after Cycle 2, cycle 3 and at the end of treatment (EOT). PhasED-seq, but not CAPP-seq, predicted event-free survival or recurrence (EFS) with 100% accuracy at EOT in this group. In a pooled analysis from 6 clinical trials, MRD- status per PhasED-seq was more prognostic than complete response (CR) by PET/CT. Patients who were already stratified per CR by PET-CT could be further stratified via PhasED-seq. 97% of patients who were tested MRD- at EOT remained disease-free long-term (median follow up was 17 months, 60 months for some patients).[23,24]

Use of point of care testing (ROTEM) in coagulopathy

Major bleeding is a frequent but often preventable cause of death after trauma or surgery. More than 80% of preventable deaths occurring in the hospital are due to hemorrhage and 25% thereof are due to coagulopathy.[25] Coagulopathy can occur during major injury with shock-induced endotheliopathy (SHINE), where large amounts of locally produced anticoagulation factors diffuse away from the wound and consume fibrinogen, thereby affecting hemostasis.[26]

Standard laboratory coagulation testing is too slow in emergencies, as every minute counts: Studies showed that a treatment delay of one minute increases mortality by 5% in patients with major bleeding.[27] Viscoelastic testing (TEG/ROTEM) of blood samples, on the other hand, yields the first results after only 10-15 minutes. TEG/ROTEM mechanically measures blood viscoelasticity during the clotting process. A pin is inserted and rotated in whole blood to register the resistance while clotting occurs. The output is a viscoelasticity curve over time. The test is performed on citrate blood with the addition of various coagulation factors and/or inhibitors. This allows a patient's coagulation to be thoroughly examined and various coagulopathies to be detected, each requiring different treatment options. Important measuring points are the viscoelasticity at 5 and 10 minutes (A5, A10), the time point of maximum clot firmness (MCF) and parameters of clot lysis (LI30, LI60, maximum lysis).[28]

The ROTEM/FIBTEM parameters MCF and A10 can predict the need for massive transfusion with sensitivities of 77.5% and 63% and specificities of 74.9% and 83.2%, respectively.[29] Fibrinogen level, in particular, is critical in patients with major bleeding and constitutes an independent predictor of patient outcome.[30] ROTEM/TEG can guide the timing of platelet, plasma and red blood cell transfusion and the needed ratio between these components.[31,32] In studies, ROTEM-guided resuscitation led to decreased mortality compared to no ROTEM guidance (7.3 vs. 13.1% overall) among trauma patients.[33]

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