EHA Perspectives on Emerging Technologies in Hematology

Presented at the EHA2024 Hybrid Congress

Madrid, Spain

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Table of contents

Welcome & Objectives

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

Objectives

This slide set highlights the Emerging Technologies in Hematology presented at the EHA2024 Hybrid Congress. It includes technologies at the forefront of gene editing, immune effector cell therapy, artificial intelligence and machine learning, new tools for diagnosis and testing, as well as targeted protein degradation. The report offers an overview of the latest innovations and their potential impact on the diagnosis, treatment, and management of hematologic conditions.

01

Gene editing

Approaches to gene editing in β-hemoglobinopathies

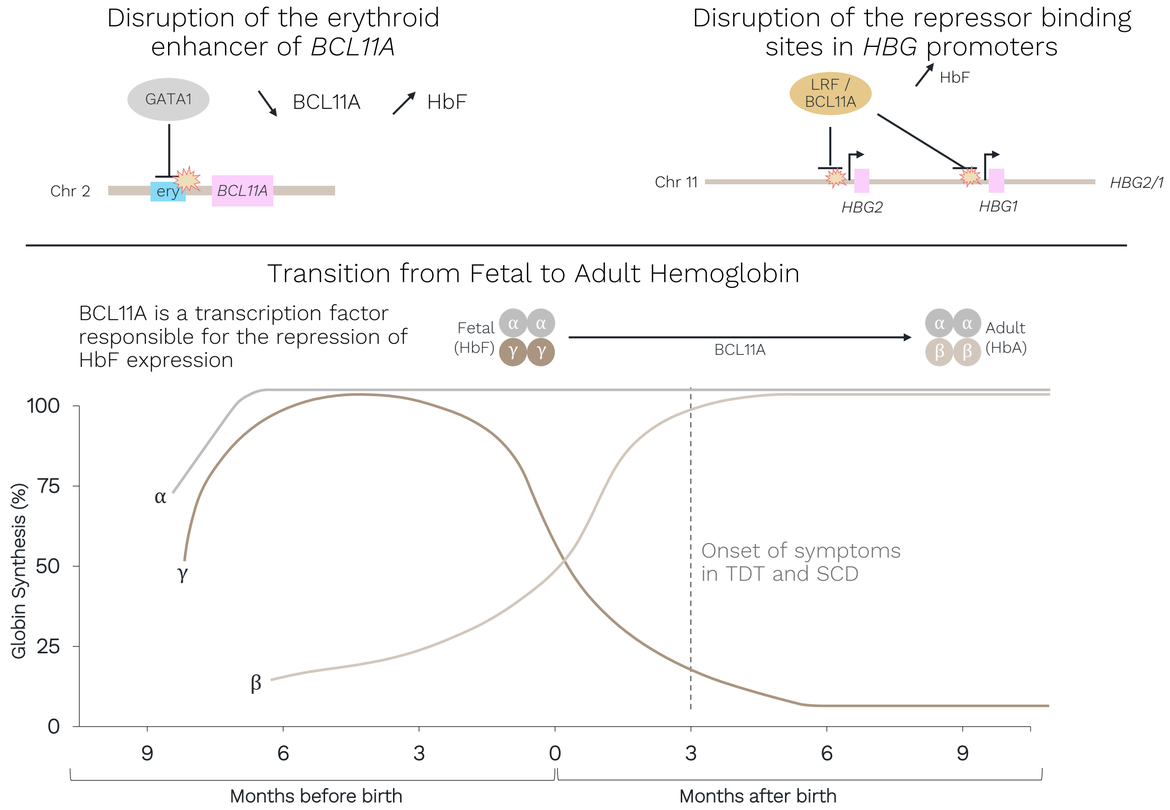


Figure adapted from Frangoul H, et al. *N Engl J Med.* 2021;384:252-260

- The two main strategies for gene editing in β-hemoglobinopathies are correcting β-globin mutations or reactivating fetal hemoglobin (HbF) by reversing its repression by either disruption of the erythroid enhancer region of *BCL11A* or editing of the BCL11A binding motif in the promoter of *HBG1/2*[1,2]
- Naturally occurring genetic variants can cause hereditary persistence of fetal hemoglobin (HPFH), leading to reduced symptoms in patients with SCD and TDT
- Gene editing strategies aim to mimic these variants in patients with β-hemoglobinopathies[3]

BCL11A, B-cell lymphoma/leukemia 11A; CRISPR, clustered regularly interspaced short palindromic repeats; HbA, hemoglobin A; HbF, fetal hemoglobin; HBG, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; SCD, sickle cell disease; TDT, transfusion-dependent thalassemia.

1. Hardouin G, et al. *Blood.* 2023;141(10):1169-1179. 2. Locatelli F, et al. *N Engl J Med.* 2024;390:1663–1676. 3. Traxler EA, et al. *Nat Med.* 2016;22(9):987-990.
Locatelli F. Clinical trials of gene editing in thalassemia. Oral presentation at EHA2024.
Hardouin G. Base-editing approaches for β-thalassemia. Oral presentation at EHA2024.

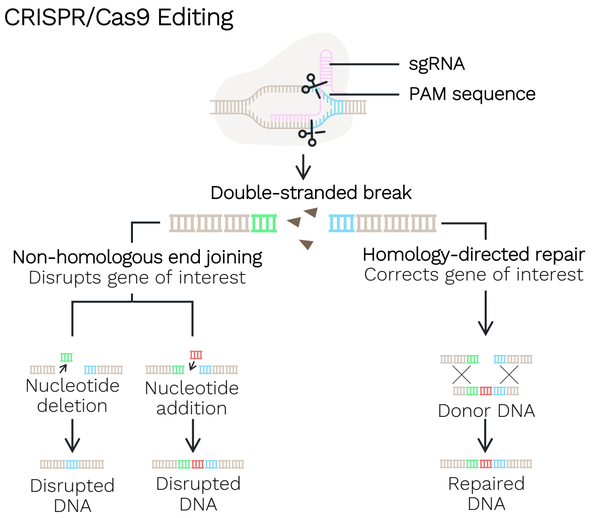
Approaches to gene editing in β-hemoglobinopathies

Two approaches to gene editing in β-hemoglobinopathies are:

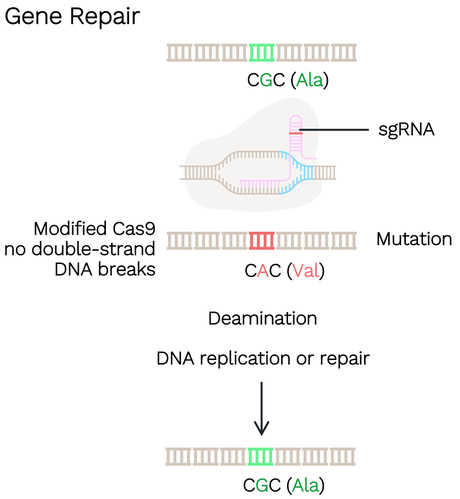
1. Gene editing or gene disruption using CRISPR/Cas9
2. Base editing using dCas9 or nCas9 fused to a deaminase

- In gene editing, CRISPR/Cas9 is used to target a specific sequence within the genome and create a DSB. The DSB can be repaired by two different pathways, NHEJ, which results in InDel formation and gene inactivation or HDR, where a donor template is provided, resulting in gene correction
  - HDR is inefficient in quiescent cells and competes with NHEJ
  - CRISPR/Cas9 can activate DNA damage repair, apoptosis, and 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[1]

1 Gene disruption or gene editing



2 Gene correction (d/nCas9)-base editing



CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; dCas9, dead Cas9; nCas9, nickase Cas9; NHEJ, non-homologous end joining; HDR, homology-directed repair; InDel, insertion/deletion; DSB, double-strand break; sgRNA, single guide RNA.

1. Rees HA & Liu DR. *Nat Rev Genet*. 2018;19:770–788.
Locatelli F. Clinical trials of gene editing in thalassemia. Oral presentation at EHA2024.
Hardouin G. Base-editing approaches for β-thalassemia. Oral presentation at EHA2024.

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

Gene editing

1. CLIMB-Thal-111 (NCT03655678) and CLIMB-121 (NCT03745287): The safety and efficacy of exa-cel for TDT and SCD were assessed in two ongoing clinical Phase 3 trials (Vertex)[1,2]
    - 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+ HSPCs
    - In patients with β-thalassemia, treatment with exa-cel resulted in transfusion independence in 91% of patients. In patients with SCD, treatment with exa-cel eliminated vaso-occlusive crises in 97% of patients for 12 months or more
    - The exa-cel safety profile is consistent with myeloproliferative busulfan conditioning and autologous HSCT.
    - Exa-cel is EMA/FDA-approved for TDT and SCD.
2. CADPT03A12101 (NCT04443907): Phase 1/2 trial to access CRISPR-Cas9-mediated disruption of the HBG1 and HBG2 gene promoters for induction of HbF in patients with severe SCD (Novartis)[3]
3. Phase 1/2 trial (NCT04211480) to assess CRISPR-Cas9-mediated disruption of the GATA1-binding site at the +58 BCL11A erythroid enhancer to induce HbF expression in children with TDT (Bioray Laboratories)[4]
4. The Ruby Trial (NCT04853576): Phase 1/2/3 study to assess the safety and efficacy of Reni-cel (AsCas12a-mediated gene editing) in SCD (see slides 9-11)

Base editing

In two pre-clinical studies, adenine base editing was used for gene correction of:

1. The *HbE* codon 26 mutation to either WT or a normal variant hemoglobin (E26G) known as Hb Aubenas (asymptomatic trait phenotype) and
2. The severe IVS1-110-(G>A) β-thalassemia mutation[5,6]
    - High base-editing efficiency was observed
    - The approach is safe as shown by transcriptome and mutation burden analysis
3. In a pre-clinical study, an adenine base editor was used to reproduce the T>C HPHF point mutation known to create a 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[7]
    - Recruitment of the KLF activator was the more potent approach
    - P53-related toxicity was relieved and fewer transcriptomic changes were seen compared to DSB-based approaches
4. First base editing clinical trial – Correctseq, CS-101: Transformer base editor for TDT
    - First patient was dosed in 2023 (see slides 12-14)

AsCas12a, Acidaminococcus sp. clustered regularly interspaced short palindromic repeats-associated protein 12a; BCL11A, B-cell lymphoma/leukemia 11A; CRISPR, clustered regularly interspaced short palindromic repeats; EMA, European Medicines Agency; exa-cel, exagamglogene autotemcel; FDA, U.S. Food and Drug Administration; HbE, hemoglobin E; HbF, fetal hemoglobin; HBG, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; HSCT, hematopoietic stem cell transplantation; KLF, Krüppel-like factor; LRF, leukemogenic transcriptional repressor factor; P53, tumor protein p53; SCD, sickle cell disease; TDT, transfusion-dependent thalassemia; WT, wild type.

1. Locatelli F, et al. *N Engl J Med*. 2024;390:1663–1676. 2. Frangoul H et al. *N Engl J Med.* 2024;390:1649–1662. 3. Sharma A, et al. *N Engl J Med.* 2023;389(9):820-832. 4. Fu B, et al. *Nat Med.* 2022;28(8):1573-1580. 5. Badat M, et al. *Nat Commun.* 2023;14(1):2238. 6. Hardouin G, et al. *Blood.* 2023;141(10):1169-1179. 7. Antoniou P, et al. *Nat Commun*. 2022;13:6618.

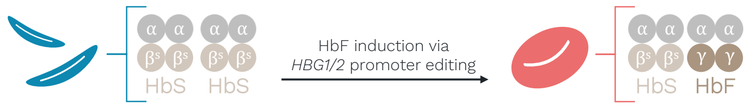
Locatelli F. Clinical trials of gene editing in thalassemia. Oral presentation at EHA2024. | Hardouin G. Base-editing approaches for β-thalassemia. Oral presentation at EHA2024.

Reni-cel: Gene editing cell therapy for sickle cell disease

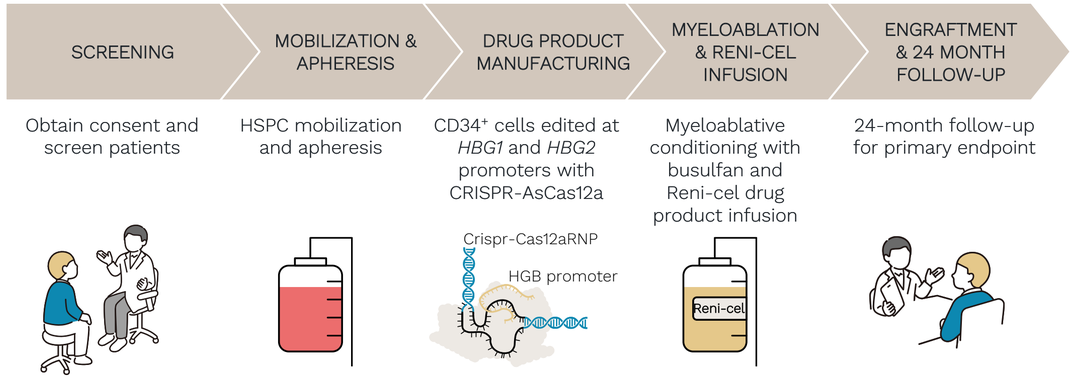
Study design and methodology

- With edits in the *HBG1* and *HBG2* promoter regions, Reni-cel mimics naturally occurring variants of HPFH to reactivate γ-globin expression and increase HbF production[1]
  - Renizgamglogene autogedtemcel (Reni-cel) is an investigational gene-edited autologous hematopoietic stem cell medicine
    - Reni-cel utilizes proprietary AsCas12a to edit with high efficiency and minimize off-target effects[2]
- The Ruby Trial (NCT04853576) is a Phase 1/2/3 international-multi-center, open-label, single-arm study to assess the safety and efficacy of Reni-cel in SCD
  - Key endpoints:
    Proportion of patients achieving complete resolution of severe vaso-occlusive events (VOEs)†
    Safety and tolerability of Reni-cel

Reni-cel mechanism of action in SCD



Study design



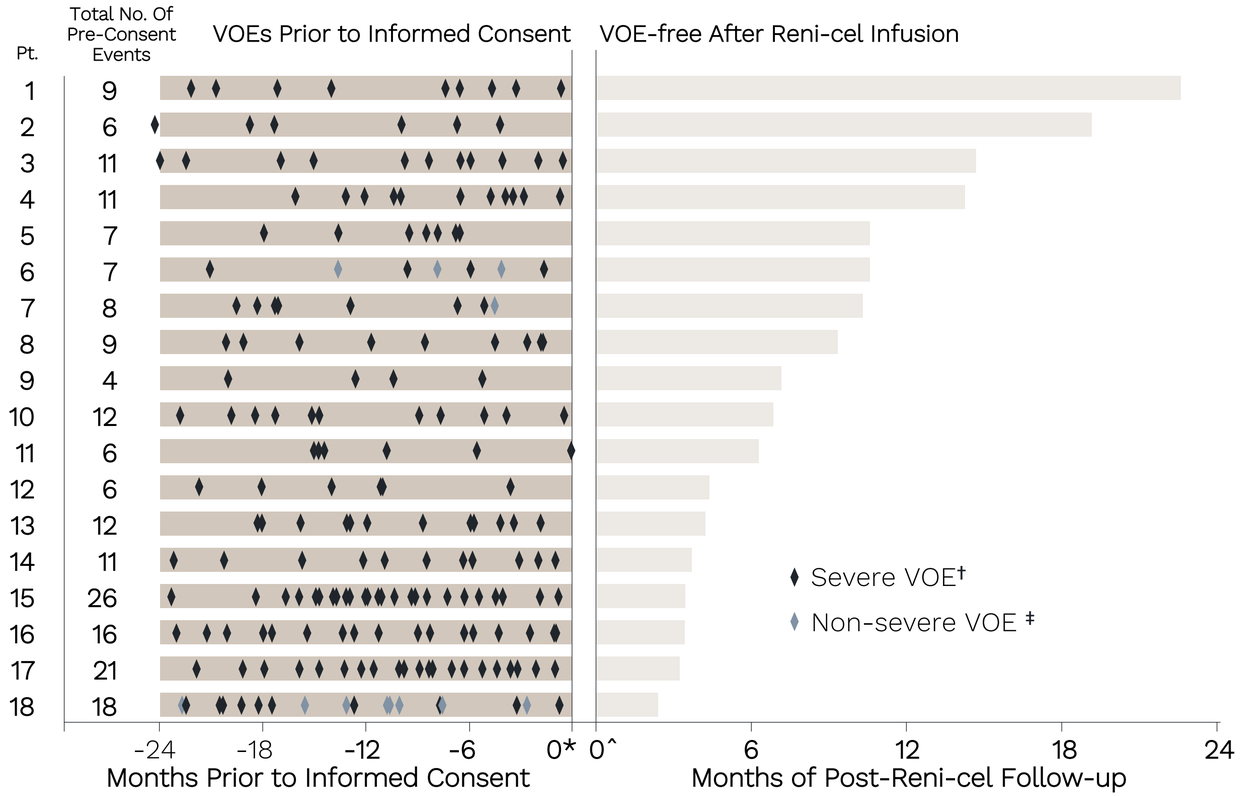
†A severe VOE requiring medical attention (despite hydroxyurea or other supportive care measures in the pre-treatment period) is defined as: an acute episode of pain with no cause other than a vaso-occlusion, resulting in either a ≥24-h hospital or Emergency Room (ER) observation unit or ≥2 visits to 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.
AsCas12a, Acidaminococcus sp. clustered regularly interspaced short palindromic repeats-associated protein 12a; CD, cluster of differentiation; HbF, fetal hemoglobin; HbS, sickle hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; RBC, red blood cell; Reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease. VOE, vaso-occlusive event.
1. Canver MC, et al. *Blood.* 2016;127(21):2536–2545. 2. Zhang L, et al. *Nat Commun.* 2021;12(1):4500.
Hanna R. Reni-cel, the first Ascas12a gene-edited cell therapy, led to hemoglobin normalization and increased fetal hemoglobin i severe sickle cell disease patients in an interim analysis of the ruby trial. Abstract S285 presented at EHA2024.

Reni-cel: Gene-edited cell therapy for sickle cell disease

Efficacy

- All patients treated with Reni-cel are VOE-free
- Patients have been VOE-free for up to 22.8 months since Reni-cel infusion
- Patients experienced rapid correction of anemia, with sustained normalization of total 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 (reticulocyte count, indirect bilirubin, LDH, and haptoglobin)

VOE after Reni-cel infusion



Left panel ends at informed consent date: 0* is day of informed consent. Right panel starts at 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.
AE, adverse event; Hb, hemoglobin; HbF, fetal hemoglobin; LDH, lactate dehydrogenase; Reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease; VOE, vaso-occlusive event.
Hanna R. Reni-cel, the first Ascas12a gene-edited cell therapy, led to hemoglobin normalization and increased fetal hemoglobin in severe sickle cell disease patients in an interim analysis of the ruby trial. Abstract S285 presented at EHA2024.

Reni-cel: Gene-edited cell therapy for sickle cell disease

Safety and tolerability

TEAE Category	N=18	Number of patients (%)	Number of events
Any TEAE		18 (100)	374
Any TEAE related to Reni-cel*		1 (5.6)	1
Any TEAE related to busulfan		18 (100)	206
Any serious TEAE†		7 (38.9)	9
Any serious TEAE related to Reni-cel		0 (0)	0
Any Grade 3 or 4 TEAE		17 (94.4)	82
Any Grade 3 or 4 TEAE related to Reni-cel		0 (0)	0
Any TEAE related to Reni-cel leading to discontinuation		0 (0)	0
Any TEAE leading to death		0 (0)	0

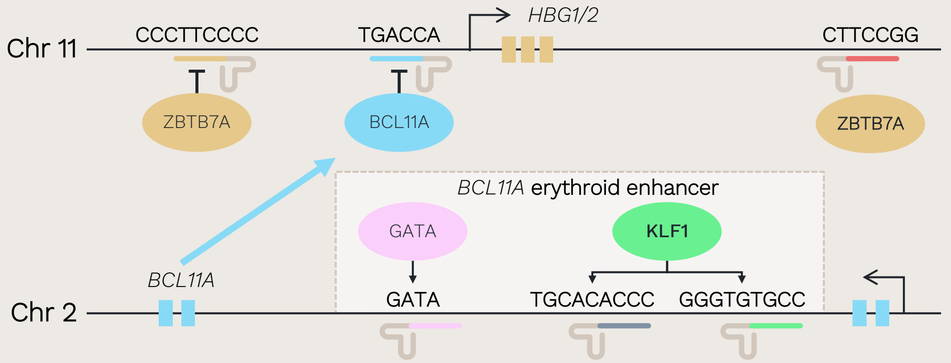
- No serious treatment-emergent adverse event (TEAEs) were reported as related to Reni-cel
- Data from treated patients demonstrated a safety profile consistent with myeloablative busulfan conditioning and autologous HSCT

Data cutoff May 8, 2024.
*One patient experienced a non-serious TEAE of Grade 1 Alanine aminotransferase increased (1.2 × ULN), which was reported to be causally related to Reni-cel and busulfan. The TEAE has resolved, and alanine aminotransferase level normalized. †As of the data cut, serious TEAEs in the RUBY trial included gastroenteritis, gastroenteritis viral, pneumonia, sepsis, chills, and hyperglycemia.
HSCT, hematopoietic stem cell transplantation; Reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.
Hanna R. Reni-cel, the first Ascas12a gene-edited cell therapy, led to hemoglobin normalization and increased fetal hemoglobin in severe sickle cell disease patients in an interim analysis of the ruby trial. Abstract S285 presented at EHA2024.

CS-101: Transformer base editor for transfusion-dependent β-thalassemia

Introduction and scientific approach

- Investigator-initiated trial of CS-101, an autologous *ex vivo* edited CD34 HSC therapy, in TDT. 6 patients have been included in the study to date.
- -114 is a naturally occurring SNV in the *HBG* promoter that leads to hereditary persistence of fetal hemoglobin (HPFH)
    - Editing of the BCL11A binding motif in the promoter of *HBG1/2* by transformer base editor (tBE) triggers more robust HbF expression than editing of the *BCL11A* erythroid enhancer by CRISPR/Cas9
- Through dual-gRNA and specific inhibitor (“lock-key”) system, tBE offers high on-target editing efficiency and eliminates off-target mutations. tBE can be delivered as mRNA/gRNA complex into the cells via *ex vivo* and *in vivo* approaches.



BCL11A, B-cell lymphoma/leukemia 11A; CD, cluster of differentiation; Chr, chromosome; CRISPR, clustered regularly interspaced short palindromic repeats; HbF, fetal hemoglobin; HBG, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; HSC, hematopoietic stem cell; SNV, single nucleotide variant; sgRNA, single guide RNA; tBE, transformer base editor.

Wang L. Treatment of patients with severe transfusion-dependent β-thalassemia with CS-101, an autologous, ex vivo edited, CD34+ hematopoietic stem cell product using innovative transformer base editor (tBE). Abstract S295 presented at EHA2024.

CS-101: Transformer base editor for transfusion-dependent β-thalassemia

Introduction and scientific approach

- To reduce off-target mutations while maintaining on-target editing efficiency, two 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
- A split-tobacco etch virus (TEV) system is employed to make the system reactive. 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

Design of the transformer base editor

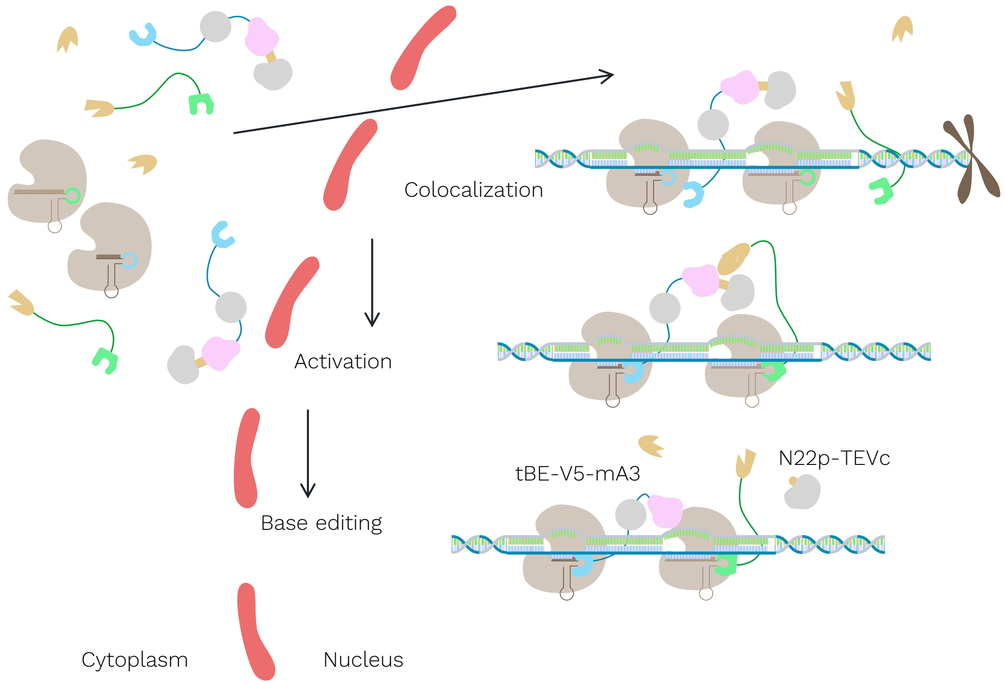
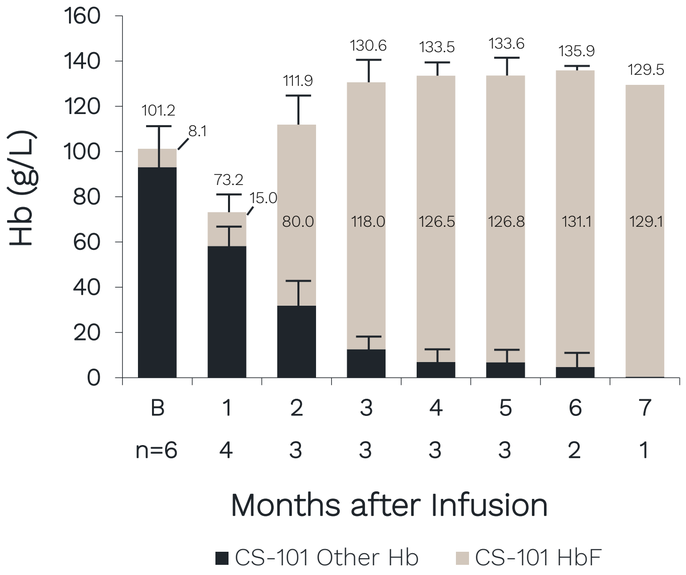


Figure adapted from Han W, et al. Cell Stem Cell. 2023;30:1624-1639 and Han W, et al. Nat Protoc. 2023;18:3194-3228.
sgRNA, single guide RNA; TEV, tobacco etch virus; tBE, transformer base editor.
Wang L. Treatment of patients with severe transfusion-dependent **β**-thalassemia with CS-101, an autologous, ex vivo edited, CD34+ hematopoietic stem cell product using innovative transformer base editor (tBE). Abstract S295 presented at EHA2024.

CS-101: Transformer base editor for transfusion-dependent β-thalassemia

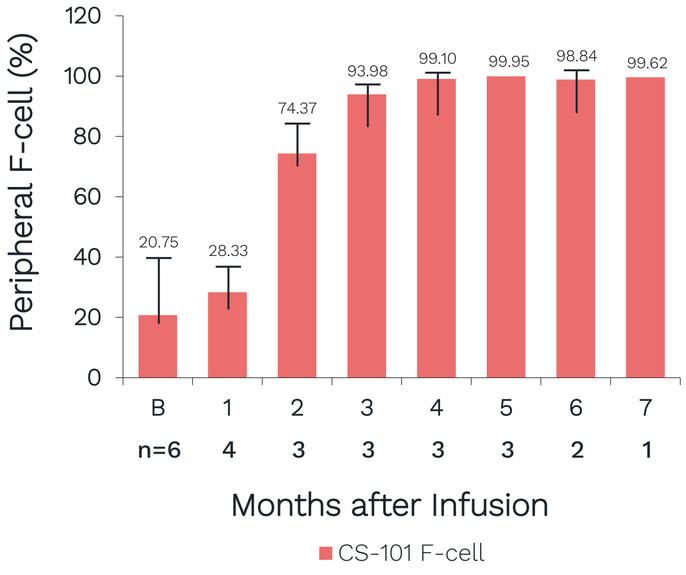
Efficacy and safety

Hemoglobin fractionation



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

F-cell



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%.

Safety

- Safety profile is consistent with autologous HSCT transplantation
- No AEs reported to be related to CS-101 up to the cutoff date
- No SAE reported up to the cutoff date

AE, adverse event; Hb, hemoglobin; HbF, fetal hemoglobin; HSCT, hematopoietic stem cell transplantation; SAE, serious adverse event.

Wang L. Treatment of patients with severe transfusion-dependent **β**-thalassemia with CS-101, an autologous, ex vivo edited, CD34+ hematopoietic stem cell product using innovative transformer base editor (tBE). Abstract S295 presented at EHA2024.

Conclusion

- Gene editing provides a potentially curative alternative to allogeneic stem cell transplantation
  - In the Ruby trial of Reni-cel in patients with SCD, all patients were VOE-free for up to 22.8 months post-infusion
  - Safety data were consistent with the myeloablative busulfan conditioning and autologous HSCT
- Base editing overcomes some of the limitations of gene editing, including DSB-induced toxicity, fewer off-target effects, no InDel formation, and greater efficiency in quiescent cells
  - First base editing clinical trial shows promising results in TDT with a safety profile consistent with autologous stem cell transplantation

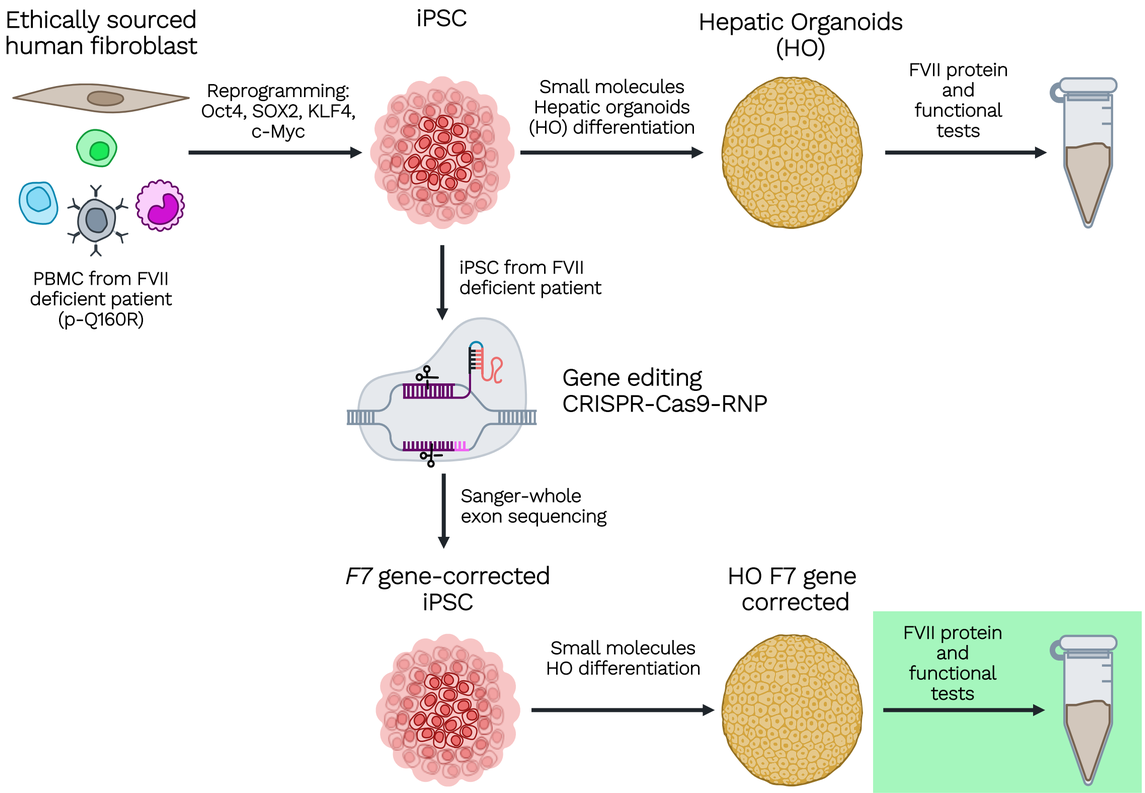
DSB, double-strand break; HSCT, hematopoietic stem cell transplantation; InDel, insertion/deletion; SCD, sickle cell disease; VOE, vaso-occlusive event.

1. Locatelli F. Clinical trials of gene editing in thalassemia. Oral presentation at EHA2024. 2. Hardouin G. Base-editing approaches for β-thalassemia. Oral presentation at EHA2024. 3. Hanna R. Reni-cel, the first Ascas12a gene-edited cell therapy, led to hemoglobin normalization and increased fetal hemoglobin in severe sickle cell disease patients in an interim analysis of the ruby trial. Abstract S285 presented at EHA2024. Wang L. Treatment of patients with severe transfusion-dependent β-thalassemia with CS-101, an autologous, ex vivo edited, CD34+ hematopoietic stem cell product using innovative transformer base editor (tBE). Abstract S295 presented at EHA2024.

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

Introduction and scientific approach

- Patients carrying *F7* p.Q160R have low FVII activity (0.6-6.5%), low FVII antigen (10-28%) and variable bleeding phenotype[1]
- Hepatic organoids derived from human iPSCs recapitulate liver biology, including the expression of coagulation factors
- 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 iPSCs
- In this pre-clinical study, *ex-vivo* editing of iPSCs from 3 patients was carried out



CRISPR, clustered regularly interspaced short palindromic repeats; c-Myc, cellular myelocytomatosis oncogene; F7, factor VII; FVII, factor VII; HO, hepatic organoid; iPSC, induced pluripotent stem cell; KLF, Krüppel-like factor; Oct4, octamer-binding transcription factor 4; PBMC, peripheral blood mononuclear cell; PSC, pluripotent stem cell; RNP, ribonucleoprotein; SOX2, SRY-box transcription factor 2.

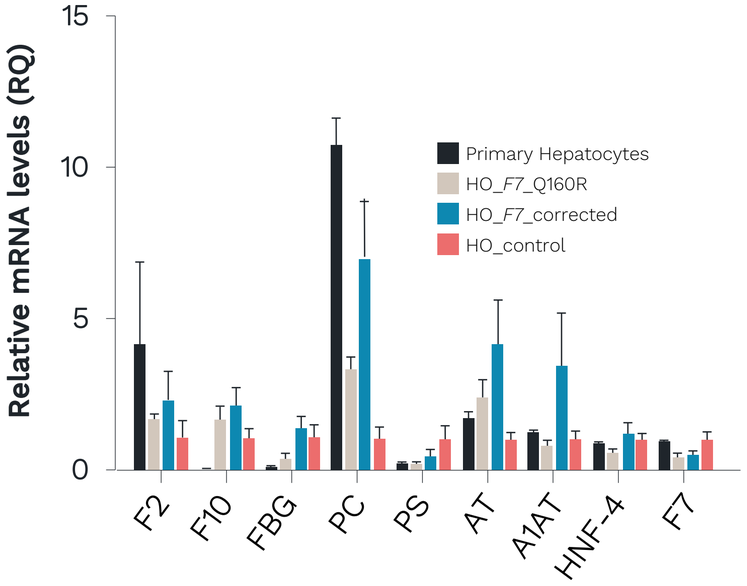
1. Kavlie A, et al. *Thromb Haemost.* 1998;79(06):1136-1143.

Chollet ME. Autologous gene-corrected stem cell-derived hepatic organoids for the treatment of FVII deficiency. Abstract S321 presented at EHA2024.

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

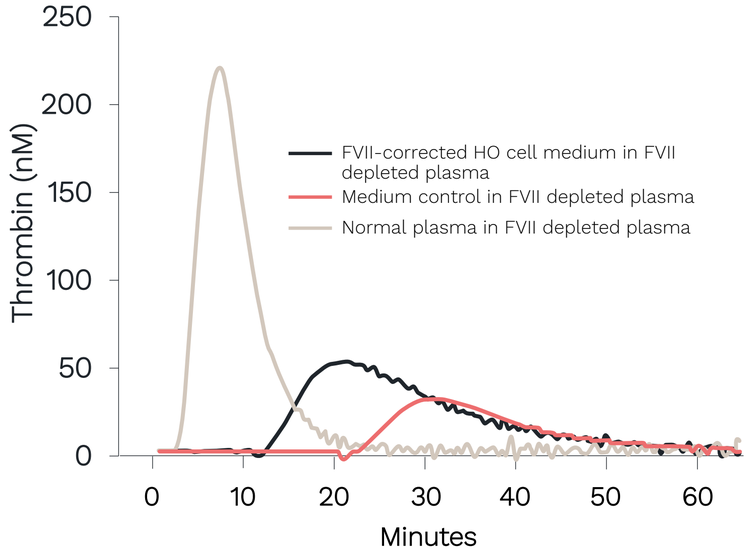
Results

Expression of coagulation factors in FVII-corrected HO



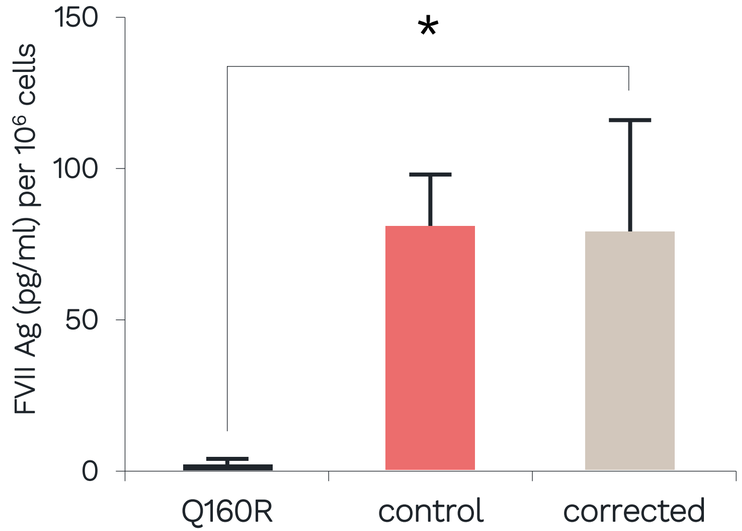
Organoids express coagulation factors at similar levels to primary hepatocytes

Thrombin generation assay



Cell medium from gene-corrected HOs shortened the lag time of FVII deficient plasma

Profiling FVII antigen and activity in p.Q160R-HO



FVII secretion into the cell medium was increased by more than 17-fold

A1AT, alpha-1 antitrypsin; Ag, antigen; AT, antithrombin; F2, factor II;, F7 factor VII; F10, factor X; FBG, fibrinogen; HO, hepatic organoid; HNF-4, hepatocyte nuclear factor 4; PC, protein C; PS, protein S; RQ, relative quantification.

Chollet ME. Autologous gene-corrected stem cell-derived hepatic organoids for the treatment of FVII deficiency. Abstract S321 presented at EHA2024.

Conclusion

- The *F7* pQ160R mutation was successfully corrected in iPSCs of 3 patients with FVII deficiency
- In this *ex vivo* study, 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

Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; FVII, factor VII; iPSC, induced pluripotent stem cell.

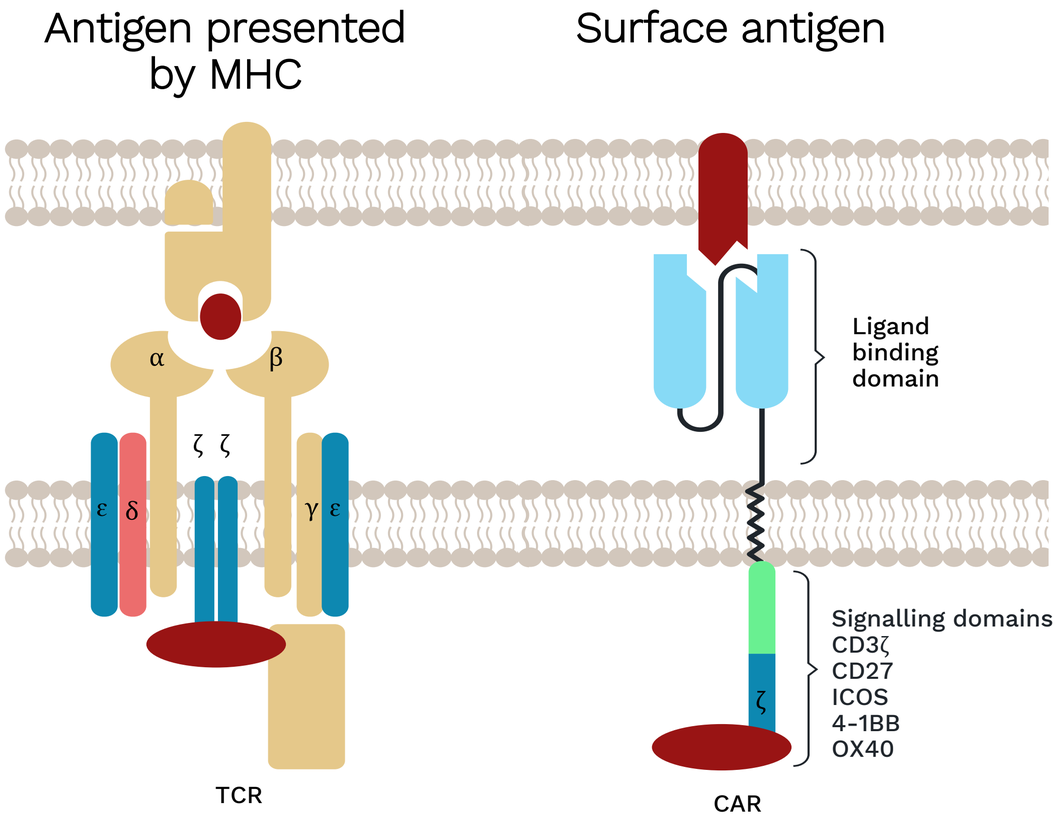
Chollet ME. Autologous gene-corrected stem cell-derived hepatic organoids for the treatment of FVII deficiency. Abstract S321 presented at EHA2024.

02

Immune effector cell therapy

CAR T and beyond

Introduction



- T-cells, engineered with chimeric antigen receptors (CAR), are the most common type of immune effector cell therapy, employed in the fight against primarily hematologic cancers
- CARs, as opposed to natural TCRs, are single molecules containing an extracellular antigen binding part and an intracellular T-cell activating domain
- Adoptive T-cells are used because endogenous T-cells against cancer antigens exist but are not abundant enough or subject to suppression
- Factors limiting the effectiveness of CAR T-cell therapies include:
  - CAR T-cells can induce toxic autoimmune effects, including CRS or ICANS[1]
  - CAR T-cells are subject to local immunosuppression in the tumor microenvironment[2]
  - Identifying specific is difficult as many possible target are also expressed in non-malignant cells
  - CAR T-cells usually target only one surface antigen; tumors can develop resistance by losing the antigen
  - Conventional CAR T-cell therapy cannot be used against T-cell leukemia/lymphoma because they share the same surface antigens

Image adapted from Garber K, et al. *Nat Biotec.* 2018; 36:215–219.
CAR, chimeric antigen receptor; CD, cluster of differentiation; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; MHC, major histocompatibility complex; TCR, T-cell receptor.
1. Garber K, et al. *Nat Biotec.* 2018; 36:215–219. 2. Kankeu Fonkoua LA, et al. Mol *Ther Oncolytics.* 2022;25:69-77.

CRISPR-based gene disruption to improve antitumor T-cell function

- Genomic sequences can be altered via classic CRISPR-Cas9, which causes DSBs, or via base editing
- How can adoptive T-cells be improved by gene editing?
  - Gene editing can make T-cells resist suppression in the tumor microenvironment, e.g. by disrupting receptors for inhibitory cytokines
  - Gene editing can enable the creation of effector cells with natural TCRs rather than CARs
- Natural TCRs have an advantage over CARs due to their higher sensitivity to tumor antigens and lower risk for ICANS.[1]

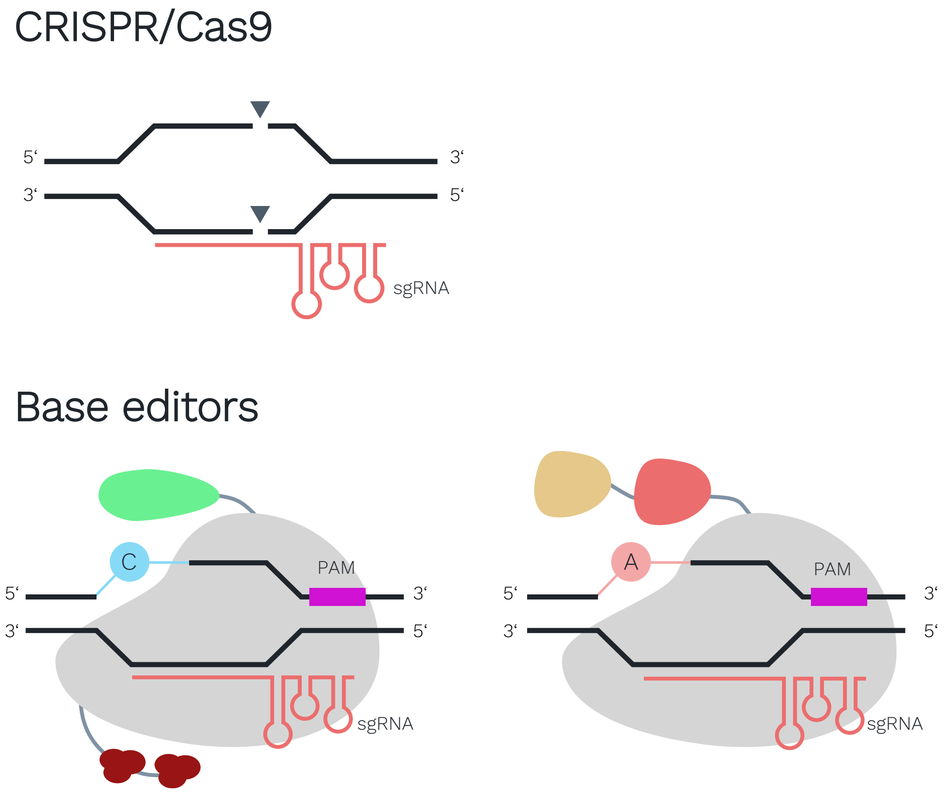


Figure adapted from Anzalone AV, et al. *Nat Biotech.* 2020; 38(7):824-844.
CAR, Chimeric antigen receptor; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; DSB, double-strand break; ICANS, immune effector cell-associated neurotoxicity syndrome; PAM, protospacer adjacent motif; sgRNA, single guide RNA; TCR, T-cell receptor.
1. Garber K, et al. *Nat Biotec.* 2018; 36:215–219.
Bonini C. CRISPR-based gene disruption to improve antitumor T cell function. Oral presentation at EHA2024.

CRISPR-based gene disruption to improve antitumor T-cell function

- Gene transfer alone is not sufficient since most cells would express multiple and hybrid TCRs
- If cells with the right TCR can be identified, they must be selected and expanded
- Alternative: Unwanted TCR is knocked out via gene editing and a new TCR is introduced via transduction
- TCRs with good anti-cancer activity can be found in cancer patients and identified in cells expressing exhaustion markers; cancer cells exposed to T-cells often have CD3 on their surface due to membrane exchange
- The option to use natural T-cell receptors in adoptive cell therapy paves the way for creating T-cell receptor libraries against different antigen targets
- Examples:
  - WT1 (antigen commonly expressed in many cancer types)[1]
  - Cathepsin G (may have role in tumorigenesis)

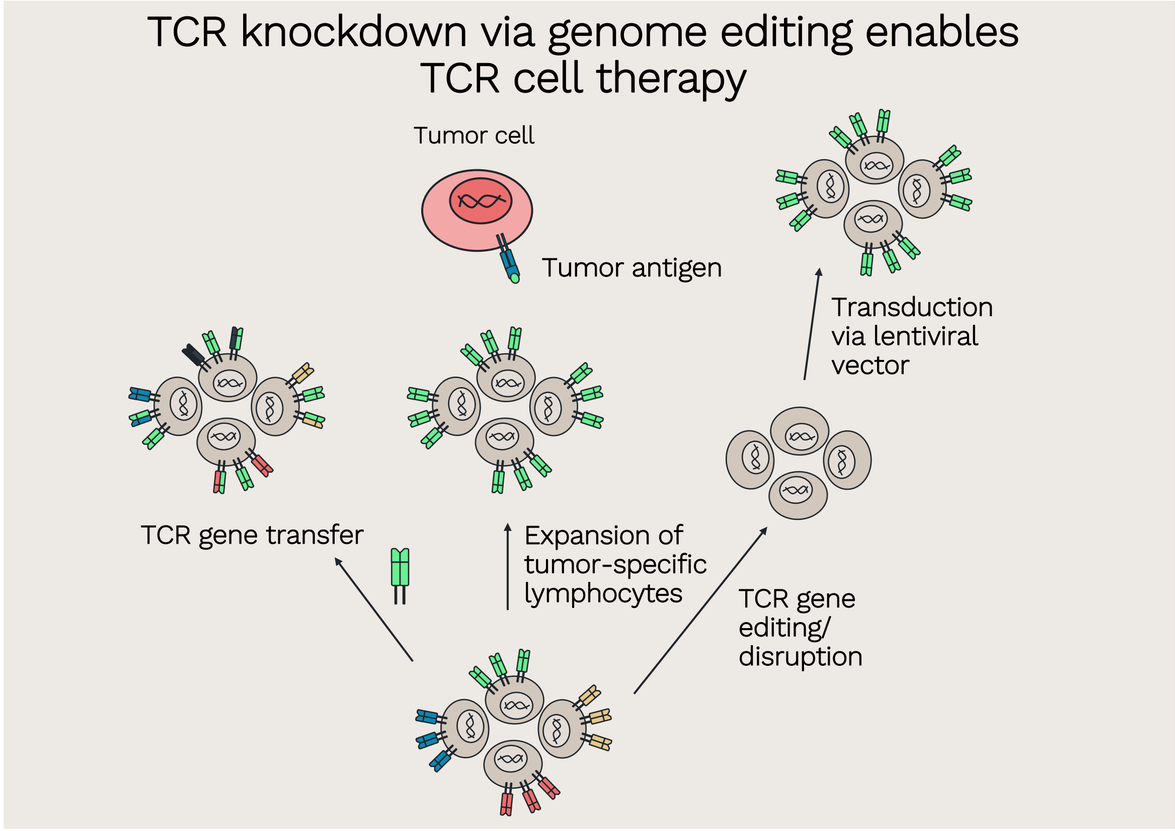
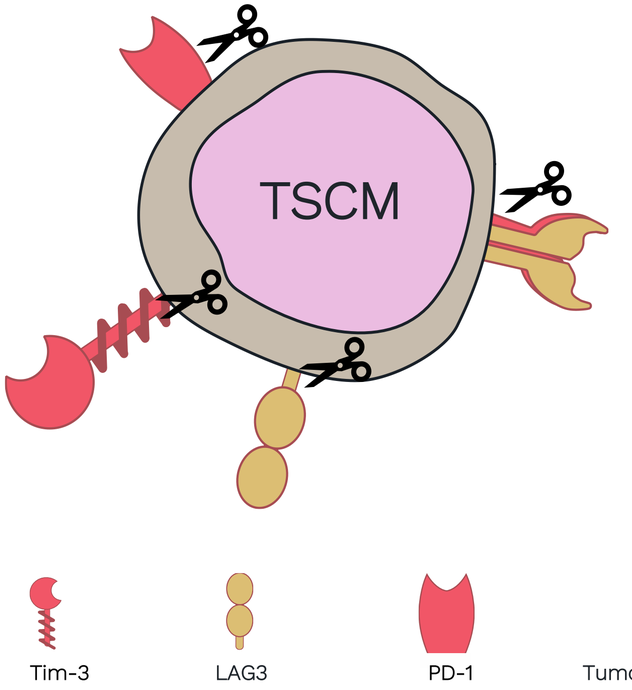


Image adapted from Provasi E, et al. *Nat Med*. 2012;18:807–815, Mastaglio S, et al. *Blood*. 2017; 130(5):606-618, and Ruggiero E, et al. *Sci Transl Med*. 2022;14(631):eabg8027.
TCR, T-cell receptor; WT, wild type.
1. Ruggiero E, et al. *Sci Transl Med*. 2022;14(631):eabg8027.
Bonini C. CRISPR-based gene disruption to improve antitumor T cell function. Oral presentation at EHA2024.

CRISPR-based gene disruption to improve antitumor T-cell function

- Gene editing can be used to make T-cells more aggressive against tumors and less prone to exhaustion and immunosuppressive environments
- How can T-cell exhaustion be overcome?
  - Checkpoint inhibition: While effective, it is associated with a high risk of toxic side effects
  - Genome editing: CRISPR-Cas9 module libraries targeting different inhibitory receptors → knock down of inhibitory receptors in the adoptive T-cells

Increasing T-cell fitness via genome editing



Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; LAG3, Lymphocyte-activation gene 3; PD-1, programmed cell death of protein 1; TCR, T-cell receptor; Tim, T-cell immunoglobulin and mucin-domain containing-3; TSCM, stem cell-like memory T-cell.
Bonini C. CRISPR-based gene disruption to improve antitumor T cell function. Oral presentation at EHA2024.

Conclusion

- CRISPR-Cas9 and base editors may be employed to improve adoptive T-cell therapy
- Cell therapy with natural TCRs is enabled by disrupting the endogenous TCR and transferring a new cancer antigen targeting TCR
- Gene editing can be used to make T-cells fitter and less susceptible to immunosuppression by knocking down inhibitory receptors

Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; TCR, T-cell receptor.
Bonini C. CRISPR-based gene disruption to improve antitumor T cell function. Oral presentation at EHA2024.

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

- Prime editing contains a modified Cas9 nickase and a reverse transcriptase instead of a nuclease and is a weakened version of CRISPR-Cas9
- The nickase creates only single-strand breaks and does not require a donor DNA, thus enabling all base exchanges + small insertions and deletions
- CRISPR prime editing is very precise and creates fewer off-target effects
- Prime editing is further improved by:
  - Engineered MLH1 protein[1]
  - Phage-assisted continuous evolution (PACE) yielding PE6 prime editors[2]

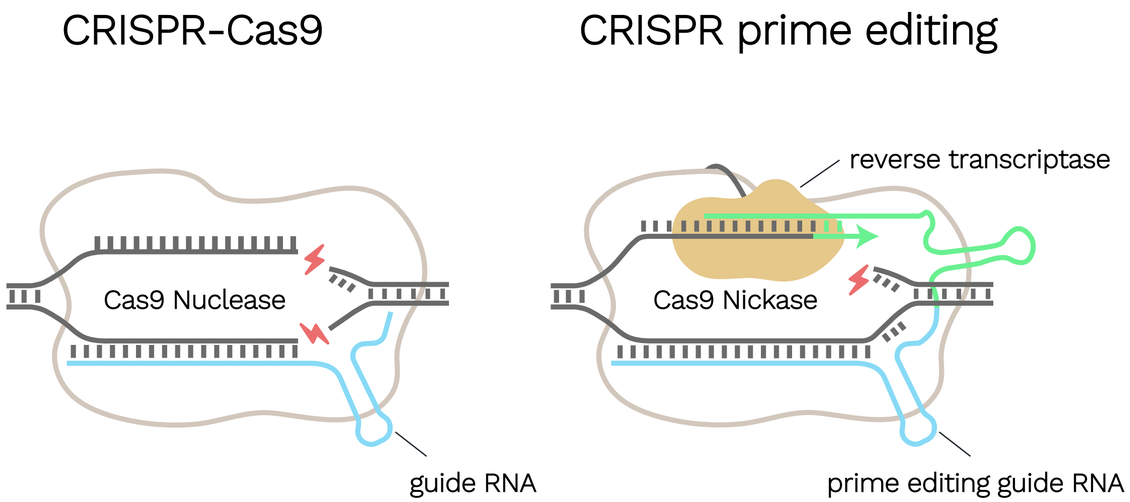
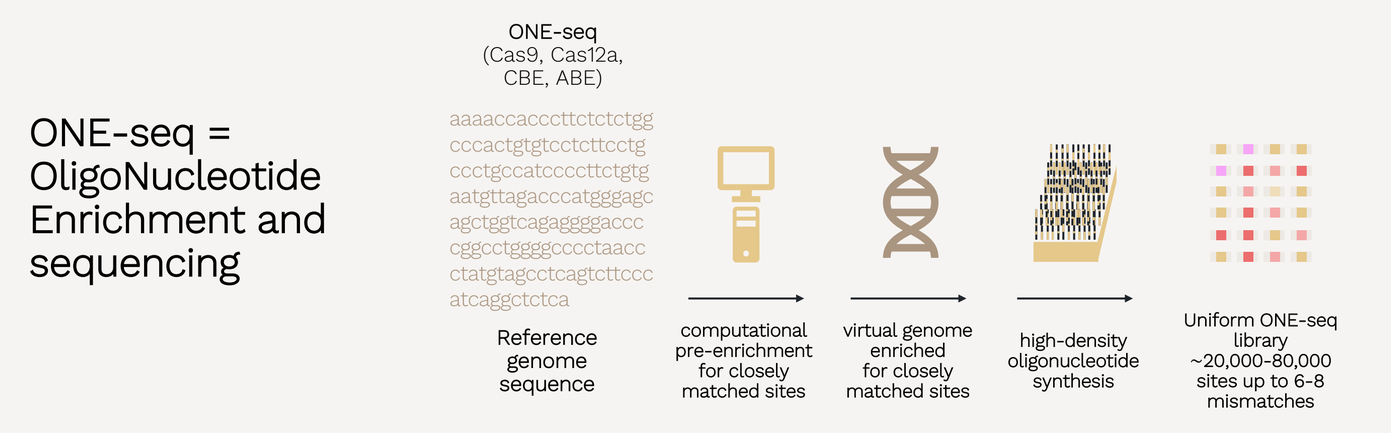
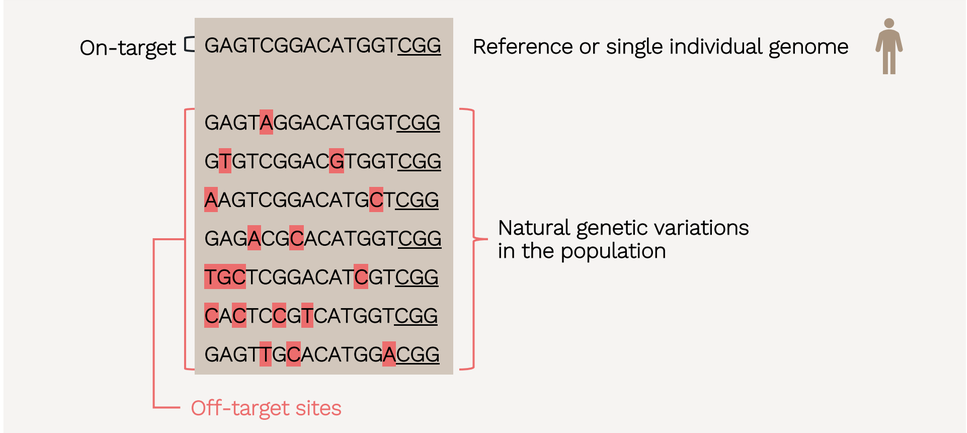


Image adapted from Jinek M, et al. *Science.* 2012;337(6096):816-821. and Anzalone AV, et al. *Nature.* 2019;576:149-157.
Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; DNA, deoxyribonucleic acid; MLH1, MutL protein homolog 1; RNA, ribonucleic acid.
1. Chen PJ, et al. *Cell.* 2021;184(22):35635-5652.e29. 2 Doman JL, et al. *Cell.* 2023;186(18):3983-4002.e26.
Petri K. Prime editing: A new tool for advanced T cell engineering. Oral presentation at EHA2024

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

- Despite its precision, CRISPR prime editing bears the risk of off-target effects.
- The safety validation can be performed by:
  - ONE-seq system for screening: Computational identification of closely matching sites, creation of synthetic DNA library followed by then *in-vitro* test with the gene editor[1]
- Natural genetic variations can vastly change the risk for off-target effects between individuals
  - Can be accounted for by including synthetic DNA with common variants





Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; ONE-seq, OligoNucleotide Enrichment and sequencing.

1. Petri K, et al. *BioRxiv.* 2021.04.05.438458.

Petri K. Prime editing: A new tool for advanced T cell engineering. Oral presentation at EHA2024.

Conclusion

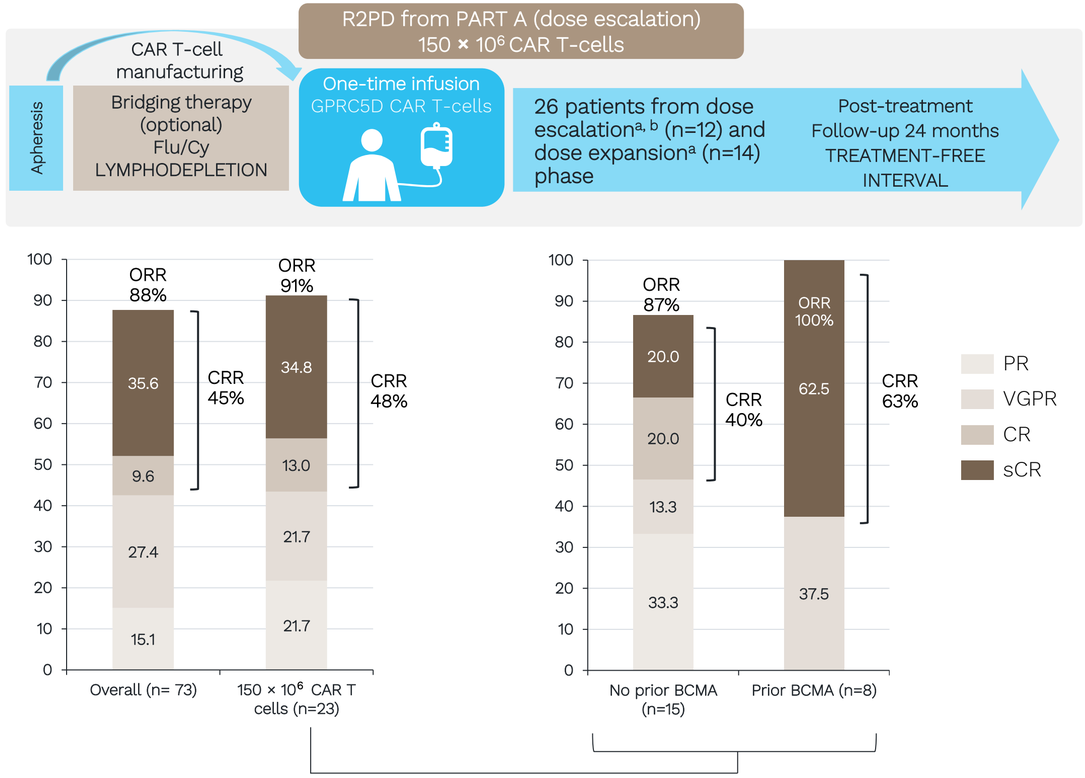
- CRISPR prime editing is a method of gene editing based on CRISPR-Cas9 that contains a modified Cas9 nickase and has fewer off-target effects compared to conventional CRISPR-Cas9
- Prime editing is a key technology in the engineering of effector cell therapies
- Off-target analysis with ONE-seq can account for the impact of natural genetic variations on off-target binding

Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; ONE-seq, OligoNecleotide Enrichment and sequencing.
Petri K. Prime editing: A new tool for advanced T cell engineering. Oral presentation at EHA2024.

Next-generation CAR T-cells in myeloma

Development of CAR T-cells with alternative antigen targets

- The most common reasons for relapse after CAR T therapy are antigen escape and antigen downregulation. Therefore, it is necessary to target additional antigens
- The surface receptor GPRC5D is a promising alternative target that is highly expressed in plasma cells but not in other immune cells or vital organs
- With Talquetamab, a GPRC5D/CD3 bispecific antibody is already approved in the treatment of MM
- GPRC5D-targeting CAR T therapy rescued mice resistant to BCMA-targeting treatment[1]
- In the first-in-human phase 1 study CC-95266-MM-001, high response rates were observed independent of prior BCMA-directed therapy[2]

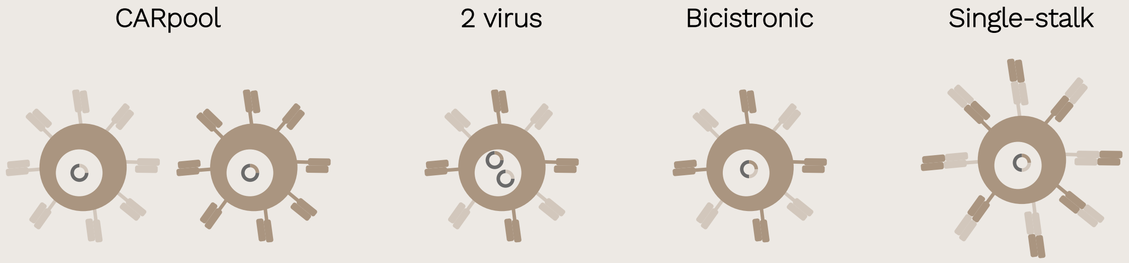


a 28-day evaluation period followed by 24 months of follow-up. b Modified toxicity probability interval (mTPI-2) design with ≥ 3 patients per dose level. 1 Smith El et al., *Sci Transl Med.* 2019 Mar 27;11(485):eaau7746. 2 Bal S et al., abstract 219, *ASH2023.* BCMA, B-cell maturation antigen; CAR T, T-cell with chimeric antigen receptor; CR, complete response; CRR, complete response rate; GPRC5D, G-protein coupled receptor family C group 5 member D; ORR, overall response rate, PR, partial response; RP2D, recommended phase 2 dose; sCR, stringent complete response; VGPR, very good partial response.
Smith E. Next generation CAR T in myeloma. Oral presentation at EHA2024.

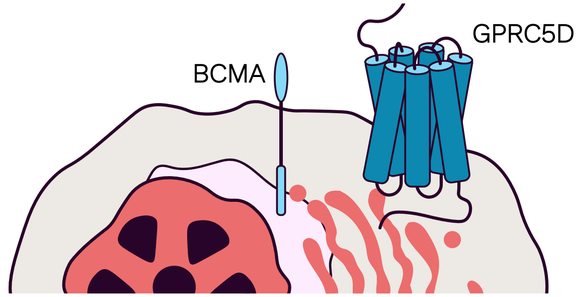
Next generation CAR T-cells in myeloma

Avoiding early resistance and post-treatment resistance to CAR T therapy

- Resistance develops due to GPRC5D loss which leads to relapse or primary refractoriness
- Dual-targeted approach: CARPOOL with two viruses with either BCMA or GPRC5D-CAR or use of a bicistronic CAR or single stalk, bispecific CAR (tandem CAR)
- The combination eliminates cells that are low or negative for either antigen (only double-negative cells can survive)
- Experiments in mice show that the single-stalk approach is superior to CARPOOL[1]



- Tumor progression MGUS → SMM → MM
- Treatment at an earlier stage of progression, e.g., SMM, might offer a chance to effectively target tumor cells before a suppressive microenvironment develops
- Early treatment has the potential to be curative
- Ciltacaptagen autoleucel is currently under evaluation for patients with high-risk SMM in the CAR-PRISM clinical trial (NCT05767359)



BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CARPOOL, pooled CAR-T-cell therapy; CAR-PRISM, CAR- PRISM (PRecision Intervention Smoldering Myeloma): A Chimeric Antigen Receptor T-cell (CAR-T) Therapy Directed Against BCMA in High-Risk Smoldering Myeloma; GPRC5D, G-protein coupled receptor family C group 5 member D; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma.

1. Fernandez de Larrea C, et al. *Blood Can Discov.* 2020;2:146-154.

Smith E. Next generation CAR T in myeloma. Oral presentation at EHA2024.

Next generation CAR T-cells in myeloma

Optimization of CAR T fitness and the manufacturing process

- Optimized CAR signaling; find clones with stronger and more durable signaling[1]
- Engineer cellular pathways to increase signaling[2,3] or fitness and survival[4,5]
- Manufacturing advances: Rapid manufacturing increases memory formation[6,7]
- Direct-to-patient CAR T-cell approaches could be even more effective[8]

In-patient CAR T-cell generation

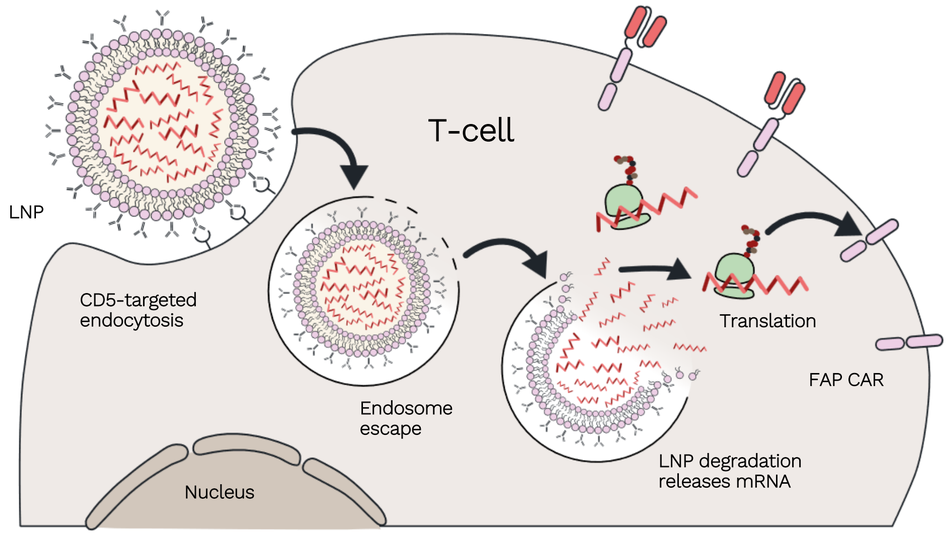


Figure adapted from Rurik J et al., *Science* 2022; 375(6576):91-96.
CAR T, chimeric antigen receptor T-cell; FAP, fibroblast activation protein; LNP, lipid nanoparticle.
1. Smith EL, et al. *Mol Ther*. 2018; 26(6):1447-1456. 2 Feucht J, et al. *Nat Med*. 2019;25(1):82-88. 3. Majzner RG, et al. *Can Discov*. 2020;10(5):702-723. 4. Lynn R, et al. *Nature*. 2019; 576(7786):293-300.
5. Labanieh L, et al. *Nature*. 2023;614:635–648. 6. Sperling AS et al., *Blood* 2021; 138:3864. 7. Ikegawa S, et al. *Blood*. 2023;142:3469–3470. 8 Rurik J et al., *Science* 2022; 375(6576):91-96.
Smith E. Next generation CAR T in myeloma. Oral presentation at EHA2024.

Conclusion

- Current T-cell redirection therapies are susceptible to a variety of pitfalls, which can be addressed with engineering or trial design approaches
- Targeting of multiple antigens (e.g. dual targeting single stalk CARs), CAR signaling optimization (e.g. knockout of inhibitory receptors) and advances in CAR T-cell manufacturing (e.g. in patient generation) are avenues to the next generation(s) of effector cell therapy
- Early-stage cancer therapy might be able to target the tumor before resistance mechanisms evolve, offering a chance for a cure to some patients

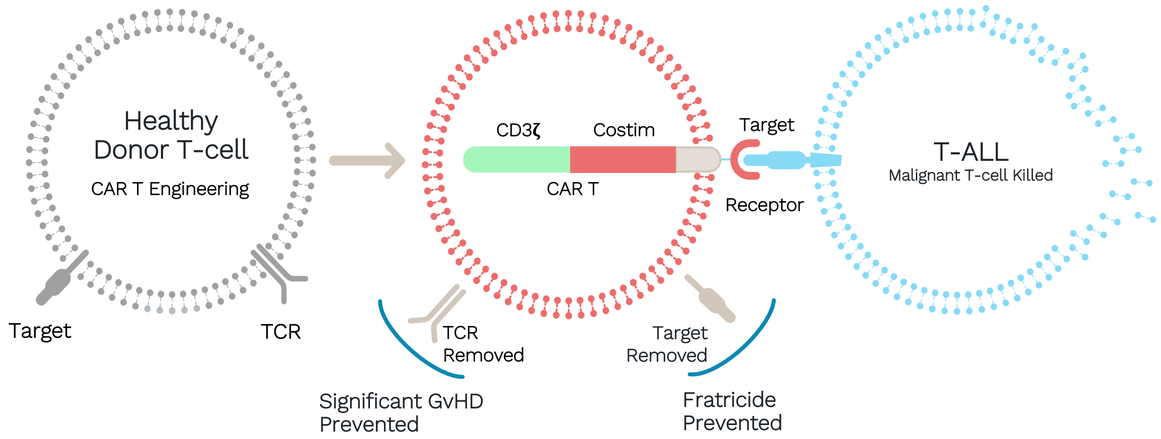
CAR T, chimeric antigen receptor T-cell.
Smith E. Next generation CAR T in myeloma. Oral presentation at EHA2024.

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

Overcoming challenges of CAR T therapy in T-cell malignancies

- R/R T-ALL/LBL are aggressive malignancies and patients have a high unmet medical need
  - CD7 is expressed in >95% of T-ALL/LBL at both diagnosis and recurrence
- Autologous CAR T-cells cannot be obtained from patients with T-cell malignancies due to the risk of contamination with malignant T-cells
- Allogeneic T-cells circumvent this risk but bear a high risk for GvHD
  - Knockout of TRAC reduces the risk of GvHD
- CD7-targeting CAR T would likely attack each other as they express low levels of CD7 themselves
  - CD7 deletion could avoid this mechanism

Wugen's Cell Engineering



CAR T, chimeric antigen receptor T-cell; GvHD, graft-versus-host disease; LBL, lymphoblastic lymphoma; R/R, relapsed/refractory; T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor; TRAC, T-cell receptor alpha constant.

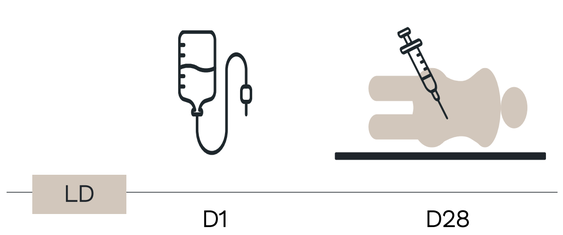
Aldoss I. WU-CART-007 (W-T7), an allogeneic CAR-T cell targeting CD7, is highly effective against relapsed/refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL). Abstract S110 presented at EHA2024.

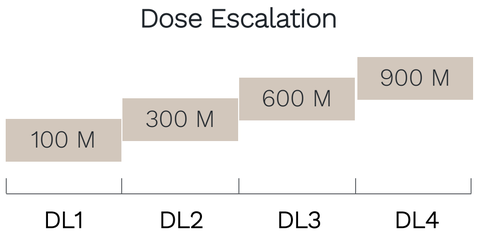
Wu-CART-007, an allogeneic CAR-T-cell therapy targeting CD7 in R/R T-ALL/LBL

WU-CART-007 1001 (NCT04984356) study design

Phase 1 – Dose escalation study

- Lymphodepletion conditioning chemotherapy (sLD):
  - Fludarabine 30 mg/m2/day x 3 (Days -5, -4, and -3)
  - Cyclophosphamide 500 mg/m2/day x3 (Days -5, -4, and -3)
- Primary objective:
  - Safety, DLT MTD/MAD
  - Define RP2D of W-T7 in T-ALL/LBL





Phase 2 – Cohort expansion study

- Enhanced lymphodepletion conditioning (eLD):
  - Fludarabine 30 mg/m2/day x 4 (Days -6, -5, -4, and -3)
  - Cyclophosphamide 1000 mg/m2/day x3 (Days -5, -4, and -3)
- Primary objective: composite complete remission rate (CRc) of W-T7 in R/R T-ALL/LBL
- Secondary objective: DOR of W-T7 in R/R T-ALL/LBL

Key eligibility criteria

- **≥12 yrs,** with evidence of **R/R* T-ALL or T-LBL, bone marrow with ≥ 5% lymphoblasts,** or evidence of **EMD**
- **ECOG** PS≤1

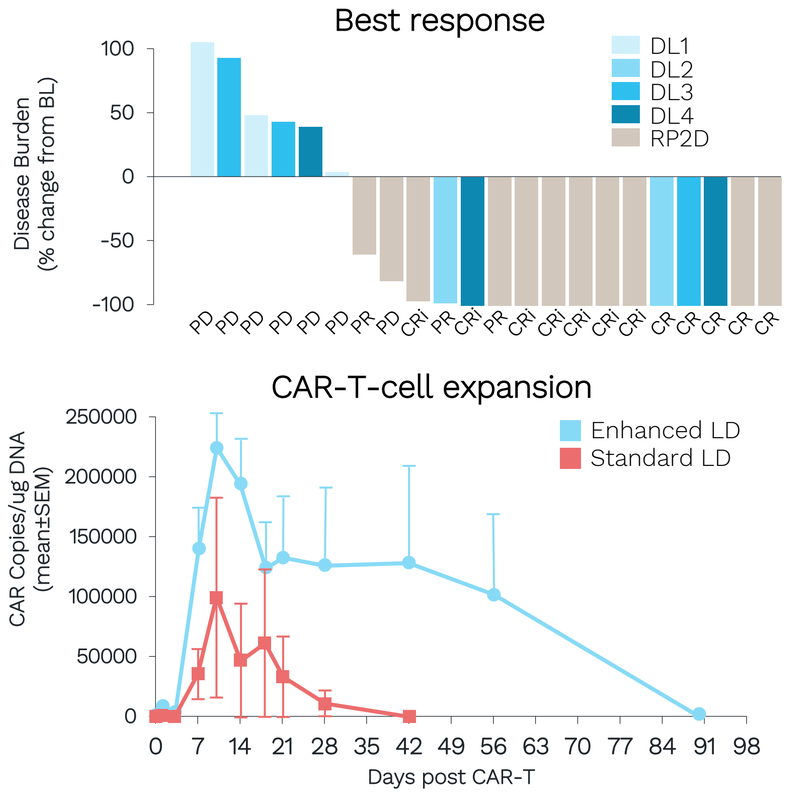
*R/R disease defined as at least one of the following criteria: Primary refractory (failure to achieve CR after induction chemotherapy), Early Relapse (relapsed disease within 12 months of initial diagnosis), Late Relapse (relapsed after 12 months of initial diagnosis + failure of reinduction therapy) or R/R disease after allogeneic transplant. D, day; DL, dose level; DLT, dose limiting toxicity; DOR, duratiuon of response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EMD, extramedullary disease; LBL, lymphoplastic lymphoma; M, million; R/R, relasped/refractory; LD, lymphodepletion; MAD, maximum administered dose; MTD, maximum tolerated dose; T-ALL, RP2D, recommended phase 2 dose; T-cell acute lymphoblastic leukemia; yrs, years.

Aldoss I. WU-CART-007 (W-T7), an allogeneic CAR-T cell targeting CD7, is highly effective against relapsed/refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL). Abstract S110 presented at EHA2024.

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

WU-CART-007 1001 efficacy results

- AT RP2D, W-T7 cell expansion peaks at day 10 and can be detected up until day 90
- No patient developed anti-drug antibodies.
- W-T7 evolve into largely CD8+ effector memory phenotype
- At RP2D, ORR was 91% (10/11) and CRc was 73% (8/11) among evaluable patients
- Median overall survival was 6.2 months



Data cut 01Jun2024. 26 pts treated; 23 pts evaluable, 3 non-evaluable (died of causes unrelated to disease progression and did not have a disease evaluation). Of evaluable pts one pt. had PD noted in CNS and is not represented on waterfall plot. Two pts. sample for MRD assessment was not available; EMD disease per Lugano criteria.
CRc, composite complete remission (CR/CRi). CAR T, chimeric antigen receptor T-cell; DL, dose level; CR(i), complete response (with incomplete count recovery); PD, progressive disease; PR, partial response; RP2D, recommended phase 2 dose.
Aldoss I. WU-CART-007 (W-T7), an allogeneic CAR-T cell targeting CD7, is highly effective against relapsed/refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL). Abstract S110 presented at EHA2024.

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

Safety

- CRS was observed in 23/26 (88.5%) patients. Most (69.2%; 18/26) patients had Gr1-2 CRS events, 5/26 (11.5%) had Gr3 CRS events or higher
- Two cases of Gr4 CRS were manageable with supportive care and completely resolved within 7 and 13 days, respectively.
- Gr1 ICANS were reported in 2 patients (7.7%) at DL3 and RP2D
- Gr2 HLH was reported in 2 patients (7.7%) at DL2 and one at the RP2D
- Gr2 GvHD was reported in 1 patient (3.8%) at RP2D
- One Gr3 prolonged cytopenia manifested as prolonged neutropenia and thrombocytopenia.
- Gr5 events were reported in three patients (11.5%); two were deemed not related (1 DL3, 1 RP2D) one (RP2D) of which was temporally related and occurred in the setting of disease progression.

Treatment-related AESI	DL4 sLD (n=3)		Expansion RP2D eLD (n=13)		All	
	Gr1-2	Gr≥3	Gr1-2	Gr≥3	Gr1-2	Gr≥3
CRS	3 (100%)	0	9 (69%)	4 (31%)	18 (69%)	5 (19.2%)
HLH	0	0	0	1 (7.7%)	1 (3.8%)	1 (3.8%)
ICANS	0	0	1 (7.7%)	0	2 (7.7%)	0
GvHD	0	0	1 (7.7%)	0	1 (3.8%)	0
Prolonged Pancytopenia[a]	0	0	1 (7.7%)[b]	1 (7.7%)	1 (3.8%)[b]	1 (3.8%)

[a]Prolonged cytopenia (including T-cell aplasia): persistent Gr≥3 cytopenia lasting more than 30 days in the absence of disease; data cutoff 23 May 2024. [b]This event was reported as Gr 2 prolonged pancytopenia; however, analysis of hematology parameters revealed persistent ≥Gr3 platelet and ANC decrease.
AESI, adverse events of special interest; ANC, absolute neutrophile count; CRS, cytokine release syndrome; DL, dose level; cytokine release syndrome; Gr, grade; GvHD, graft-versus-host disease; HLH, hemophagocytic lymph histiocytosis; ICANS, immune effector cell-associated neurotoxicity syndrome; RP2D, recommended phase 2 dose.

Aldoss I. WU-CART-007 (W-T7), an allogeneic CAR-T-cell targeting CD7, is highly effective against relapsed/refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL),.Abstract S110, presented at EHA2024.

Conclusion

- The CD7-targeting, WT7-deleted WU-CART-007 CAR T-cell therapy overcomes key challenges to the use of CAR T-cells in T-ALL/LBL
- The therapy is effective in a R/R patient population with 100% ORR and 67% CR/CRi at RP2D
- Safety is manageable, with CRS being the most common Gr≥3 TRAE, affecting 31% of patients at RP2D
- New P2 pivotal study WUC007-03 study in T-ALL/LBL on patients ≥1 y with evidence of R/R disease is underway

CAR T, chimeric antigen receptor T-cell; CRS, cytokine release syndrome; LBL, lymphoplastic lymphoma; ORR, overall response rate; RP2D, recommended phase 2 dose; R/R, relapsed/refractory; T-ALL, T-cell acute lymphoblastic leukemia; TRAC, T-cell receptor alpha constant; TRAE, treatment-related adverse event.

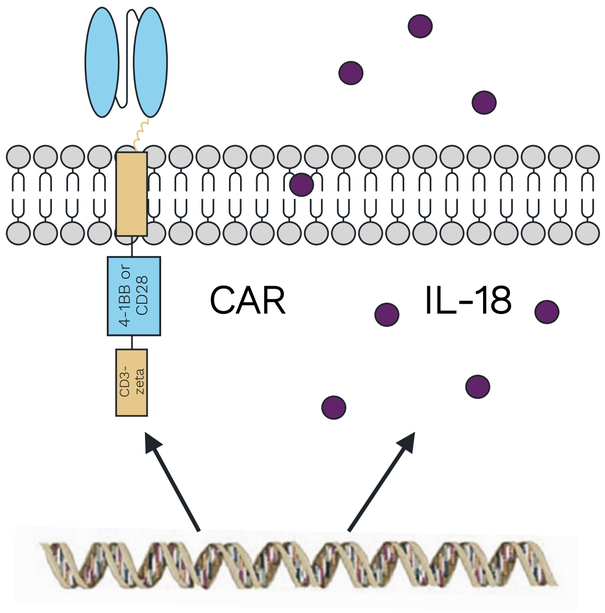
Aldoss I. WU-CART-007 (W-T7), an allogeneic CAR-T-cell targeting CD7, is highly effective against relapsed/refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL),.Abstract S110 presented at EHA2024.

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

Interleukin 18 is a proinflammatory cytokine increasing the anti-tumor activity of T-cells

- IL-18 enhances cytolytic potential, modulates the tumor microenvironment and has anti-lymphoma effects
- First-in-human trial using an armored CAR T-cell product with the capacity to secrete transgenic IL-18 (a fourth-generation CAR)
  - Autologous 4-1BB CAR-T targeting CD19
  - Humanized version of scFv
  - Expedited 3-day manufacturing
- Armored huCART19-IL18 was evaluated in B-cell lymphoma patients who progressed after currently available anti-CD19 CAR T

Fourth-generation CAR (“Armored CAR”)



CAR T, chimeric antigen receptor T-cell; R/R, relapsed/refractory; scFv, single chain variable fragment.

Svoboda J. Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas who progressed after anti-CD19 CAR T-cell therapy. Abstract S286, presented at EHA2024.

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

Study design

Screening ▶ Apheresis Manufacturing ▶ Bridging Therapy (optimal) ▶ Baseline Imaging ▶ LD Chemo (last dose D-2) ▶ **huCART19-IL18 (D0)** ▶ Tumor Biopsy (D14) ▶ Response Assessment M 3, 6, 9, 12

Key inclusion criteria (NHL cohort)
- ≥18 years old
- Documentation of CD19
- At least 2 lines of prior therapy
- Must be relapsed/refractory to prior CAR
- ECOG PS 0-1

Key exclusion criteria
- Active CNS disease
- Active autoimmune disease

Modified Bayesian optimal interval dose titration
Re-treatment was permitted in non-CR patients

Lymphodepleting chemotherapy

Cyclophosphamide 250 mg/m^2
+ Fludarabine 25 mg/m^2 x 3 days

or

Bendamustine 90 mg/m^2 x 2 days

Dose level (DL)	huCART19.IL18+ cells	LD Chemo
DL-1	7x10^5 cells	No
DL1A*	**3x10^6 cells**	**No**
DL1B	3x10^{6}cells	Yes
DL2	7x10^{6}cells	Yes
DL3	3x10^{7}cells	Yes
DL4	7x10^{7}cells	Yes
DL5	3x10^{8}cells	Yes

*starting dose

PRIMARY OBJECTIVE

Safety

SECONDARY OBJECTIVES

Manufacturing feasibility

Preliminary efficacy

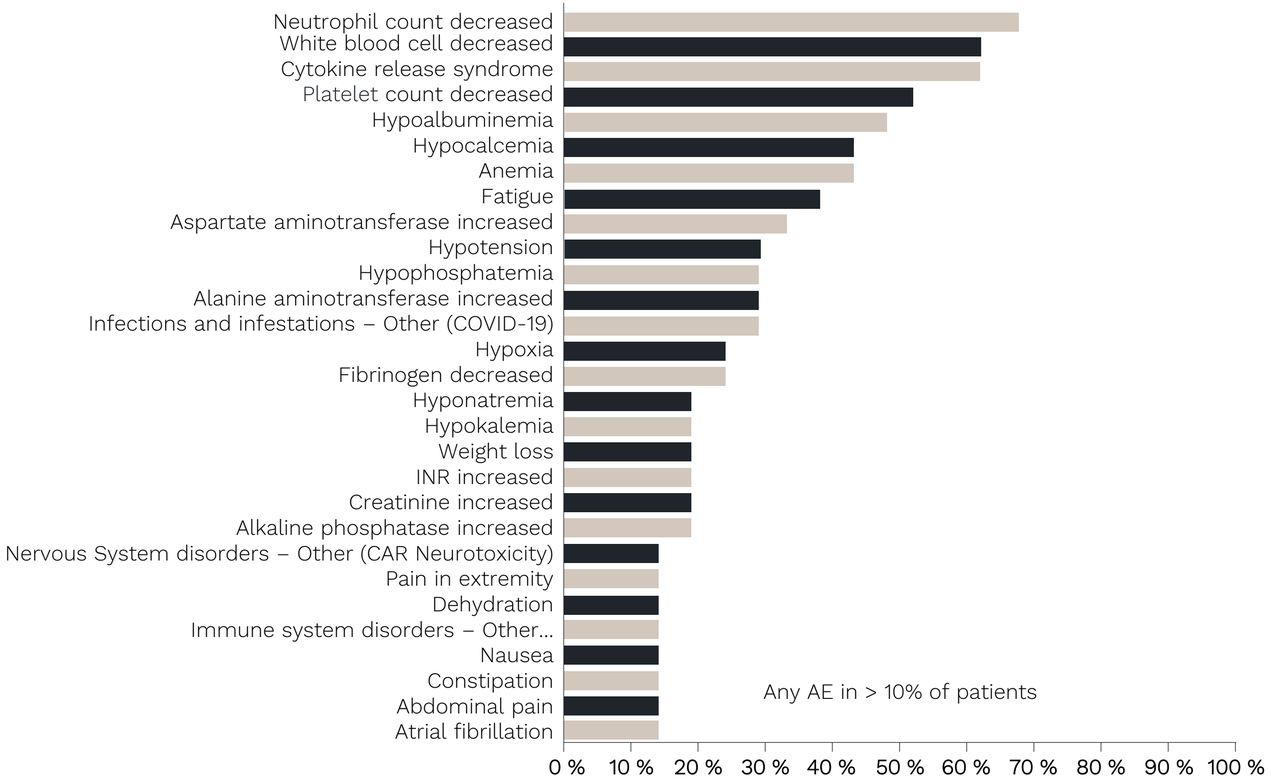
Correlative studies

CAR T, chimeric antigen receptor T-cell; CNS, central nervous system; CR, complete response; D, day; DL, dose level; ECOG PS, Eastern Cooperative Oncology Group performance status; LD, lymphodepletion; NHL, non-Hodgkin's lymphoma; R/R, relapsed/refractory.

Svoboda J. Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas who progressed after anti-CD19 CAR T-cell therapy. Abstract S286, presented at EHA2024.

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

Safety summary



Toxicities of special interest

CRS	**Any grade**	**13 (62%)**
	Grade 1	7 (33%)
	Grade 2	3 (14%)
	Grade 3	3 (14%)
ICANS	**Any grade**	**3 (14%)**
	Grade 1	2 (10%)
	Grade 2	1 (5%)
	Grade 3	0

MEDIAN ONSET
day 4 (1–11)

MEDIAN DURATION
7 days (3–12)

MEDIAN ONSET
day 8 (7–20)

MEDIAN DURATION
7 days (3–7)

AE, adverse event; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; R/R, relapsed/refractory.

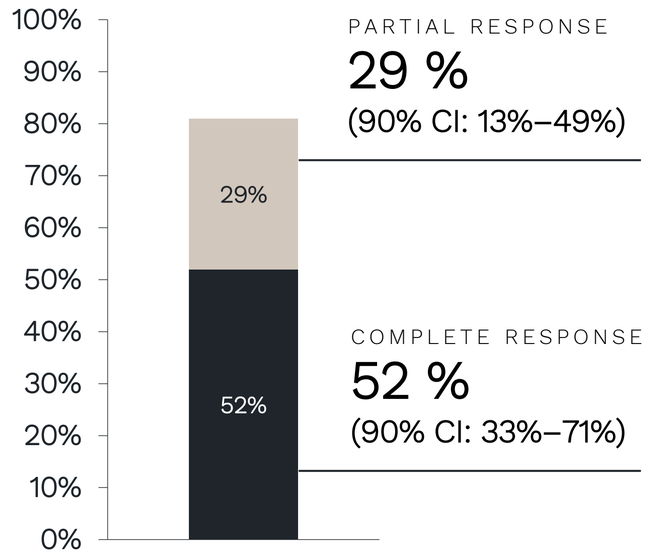
Svoboda J. Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas who progressed after anti-CD19 CAR T-cell therapy. Abstract S286 presented at EHA2024.

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

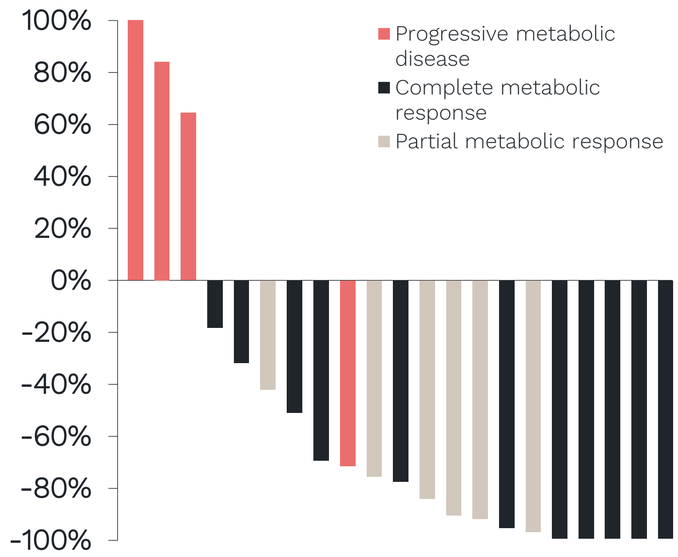
Efficacy data

Responses at 3 months

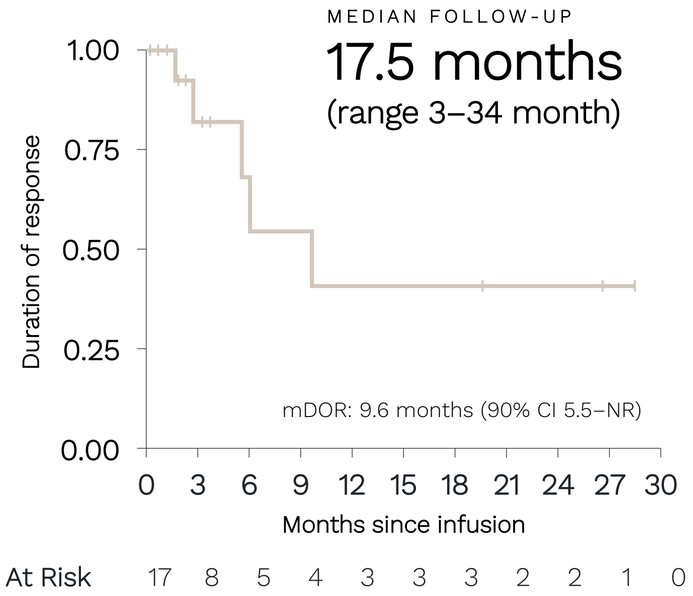
Overall response rate: 81% (90% CI: 62–93%)



Tumor volume change (%)



Duration of response mDOR: 9.6 month (90% CI: 5.5-NR)



CI, confidence interval; mDOR, median duration of response; NR, not reached.

Svoboda J. Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas who progressed after anti-CD19 CAR T-cell therapy. Abstract S286 presented at EHA2024.

Conclusion

- huCART19-IL18 is an “armored” CAR-T cell product secreting proinflammatory IL-18
- Treatment with huCART19-IL18 is feasible and did not raise unexpected safety concerns
- huCART19-IL18 results in durable responses even after prior failure of anti-CD19 CAR-T therapy
- Preliminary correlative studies suggest that IL-18 enhances CAR T-cell efficacy through intrinsic/extrinsic mechanisms

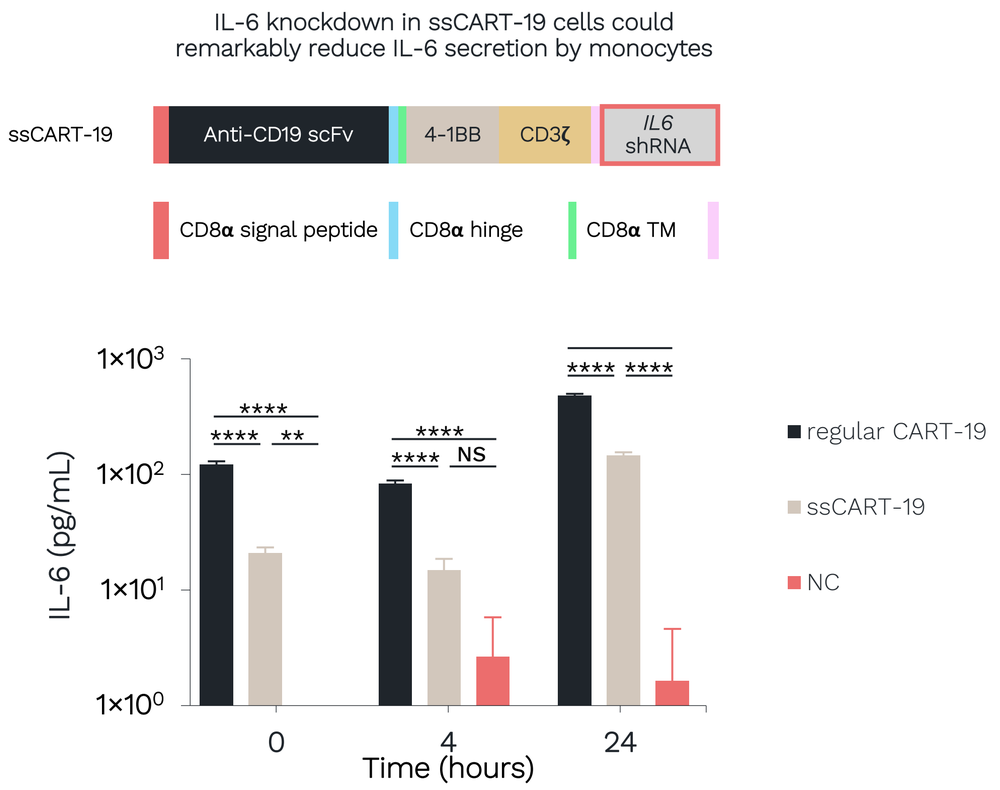
CAR T, chimeric antigen receptor T-cell.

Svoboda J, Safety and efficacy of armored huCART19-IL18 in patients with relapsed/refractory lymphomas who progressed after anti-CD19 CAR T-cell therapy, Abstract S286, presented at EHA2024

High efficacy and safety of IL-6-knockdown CD 19-targeted CAR T-cells in R/R B-ALL patients

IL-6 mediated CAR-T toxicity could be alleviated via *IL-6* silencing

- Innovative CAR structure design of ssCART 19: Anti-CD19 CAR-T with an *IL-6* silencing element (shRNA insertion)
- ssCART-19 can simultaneously express CAR structures and the *IL-6* silencing element, controlling the overall release intensity of cytokines
- The shRNA insertion did not affect CAR T amplification
- Hypothesis: ssCART-19 could mitigate severe CRS while preserving the available anti-tumor effect in patients with R/R B-ALL

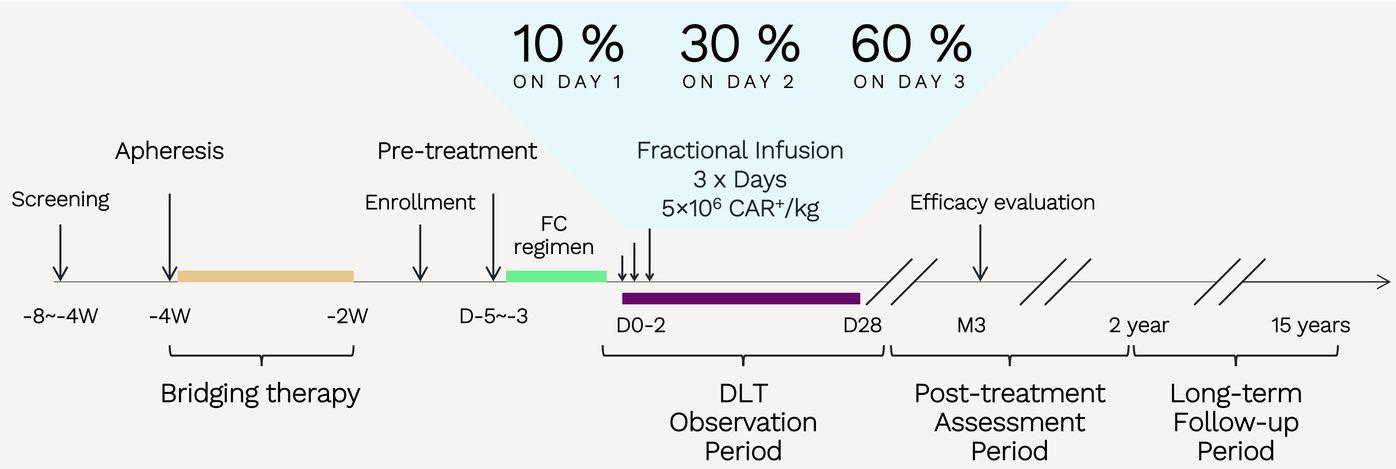


Figures adapted from Kang L et al., xp *Hematol Oncol.* 2020; Jun 8:9:11.
B-ALL, B-cell acute lymphocytic leukemia; CAR T, chimeric antigen receptor T-cell; CRS, cytokine release syndrome; NC, negative control; NS, not significant; R/R, relapsed/refractory; scFv, single chain variable fragment; shRNA, small hairpin RNA; ssCART-19.
Yu L. High efficacy and safety of interleukin-6-Knockdown CD19-targeted CAR-T-cells in relapsed/ refractory B-ALL patients. Abstract S287 presented at EHA2024

High efficacy and safety of IL-6-Knockdown CD-19-targeted CAR-T-cells in R/R B-ALL patients

Study design

- Single-arm, open-label study conducted at two centers (NCT03919240)
- Patient population: Patients with R/R B-ALL (all ages)
- Primary endpoint: Rate of CRS
- Secondary endpoints: CR/CRi, PFS, OS
- Exploratory objectives: Pharmacokinetics, pharmacodynamics



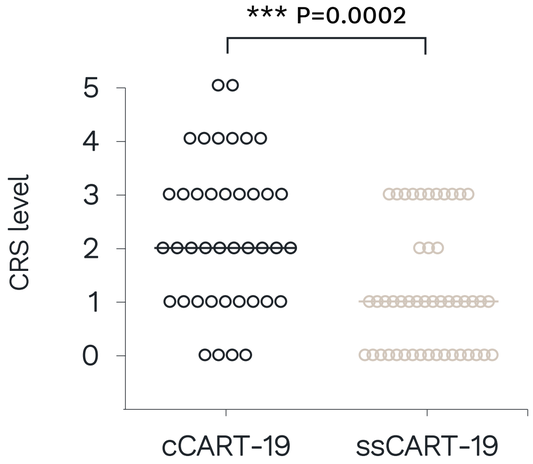
B-ALL, B-cell acute lymphocytic leukemia; CR(i), complete response (with incomplete count recovery); CRS, cytokine release syndrome; DLT, dose limiting toxicity; FC, fludarabine and cyclophosphamide; OS, overall survival; PFS, progression-free survival; R/R, relapsed/refractory.

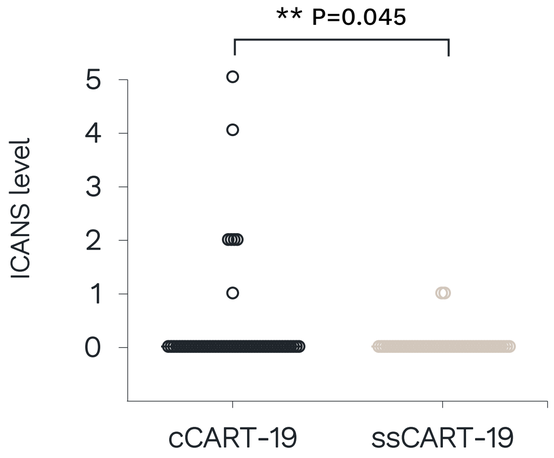
Yu L. High efficacy and safety of interleukin-6-Knockdown CD19-targeted CAR-T cells in relapsed/ refractory B-ALL patients. Abstract S287 presented at EHA2024.

High efficacy and safety of IL-6-Knockdown CD-19-targeted CAR-T-cells in R/R B-ALL patients

Therapy toxicity

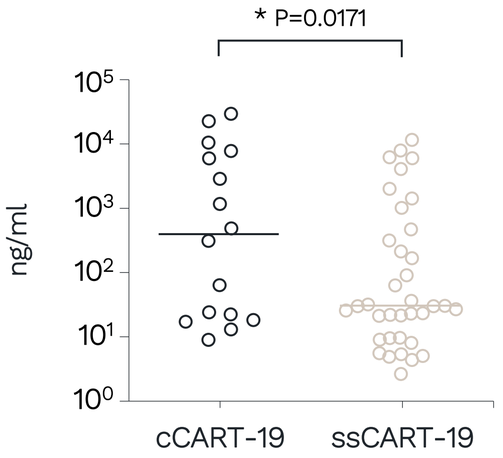
	ssCART-19(n=47)		cCART-19(n=40)	
	Any grade	Grade≥3	Any grade	Grade≥3
CRS	32 (68.09%)	7 (14.89%)	34 (85%)	15 (37.5%)
ICANS	2 (4.26%)	0 (0%)	6 (15%)	2 (5%)





IL-6 level

Rates and severity of CRS and ICANS, as well as peak levels of IL-6, IL-2 and TNFα, were significantly lower in the ssCART-19 group compared to the cCART-19 group



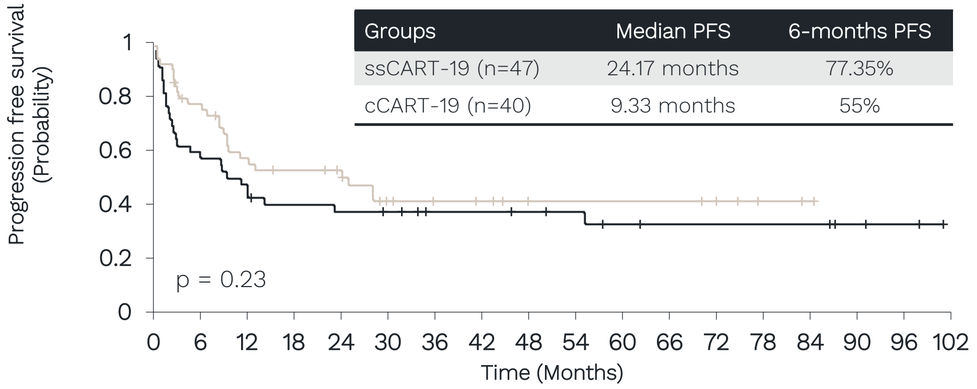
cCART, classical chimeric antigen receptor T-cell; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; ssCART, small hairpin RNA element to silence the interleukin-6 (IL-6) gene chimeric antigen receptor T-cell; TNFα, tumor necrosis factor alpha.

Yu L. High efficacy and safety of interleukin-6-Knockdown CD19-targeted CAR-T cells in relapsed/ refractory B-ALL patients. Abstract S287 presented at EHA2024.

High efficacy and safety of IL-6-knockdown CD-19-targeted CAR-T-cells in R/R B-ALL patients

Efficacy data

Progression-Free Survival (PFS)



Groups	Median PFS	6-months PFS
ssCART-19 (n=47)	24.17 months	77.35%
cCART-19 (n=40)	9.33 months	55%

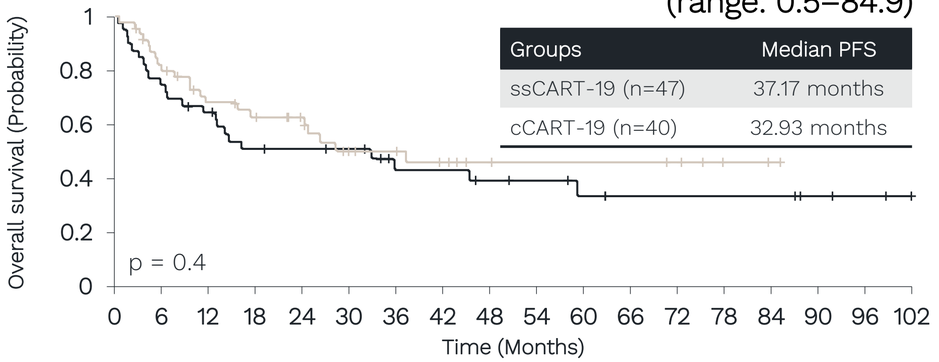
Number at risk																		
cCART-19	40	23	19	15	14	13	10	10	9	8	6	5	5	5	5	3	2	0
ssCART-19	47	35	25	22	19	12	10	9	7	6	6	6	5	2	1	0	0	0

Overall Survival (OS)

MEDIAN FOLLOW-UP TIME

21.9 months

(range: 0.5–84.9)



Groups	Median PFS
ssCART-19 (n=47)	37.17 months
cCART-19 (n=40)	32.93 months

Number at risk																		
cCART-19	40	30	25	19	18	16	11	11	9	8	6	5	5	5	5	3	2	0
ssCART-19	47	36	28	25	21	14	12	10	7	6	6	6	5	2	1	0	0	0

Response (within 3 months)	ssCART-19 (n=47)	cCART-19 (n=40)
ORR (CR+Cri), n(%)	43 (91.49%)	34 (85.0%)

ssCART-19
cCART-19

cCART, classical chimeric antigen receptor T-cell; CR(i), complete response (with incomplete count recovery); ORR, overall response rate; ssCART, small hairpin RNA element to silence the interleukin-6 (IL-6) gene chimeric antigen receptor T-cell.

Yu L. High efficacy and safety of interleukin-6-Knockdown CD19-targeted CAR-T cells in relapsed/ refractory B-ALL patients. Abstract S287 presented at EHA2024.

Conclusion

- ssCART-19 is a CAR-T product with attenuated autoreactive response, thanks to partial *IL-6* silencing
- The study results demonstrate the safety and efficacy of ssCART-19 against R/R B-ALL
- Patients who received ssCART-19 experienced CRS and ICANS at lower rates and severity than patients receiving cCART-19
- Response rates and survival were numerically better (but not statistically significant) in patients receiving ssCART-19 vs. cCART-19

B-ALL, B-cell acute lymphocytic leukemia; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; PFS, progression-free survival; R/R, relapsed/refractory.

Yu L. High efficacy and safety of interleukin-6-Knockdown CD19-targeted CAR-T cells in relapsed/ refractory B-ALL patients, Abstract S287 presented at EHA2024

03

Multi-omics

Introduction

- Omics technologies have significantly contributed (and continue to contribute) to a better understanding of hematological malignancies
- No single technology can offer all the necessary information
- By combining omics technologies, we can now obtain different levels of information (spatial, cellular, molecular, etc.) that provide a clearer picture of the complex mechanisms underlying hematological malignancies
- Omics-based approaches for diagnosis, disease monitoring, or predicting treatment response could play a pivotal role in clinical decision-making and potentially lead to more personalized and effective patient care

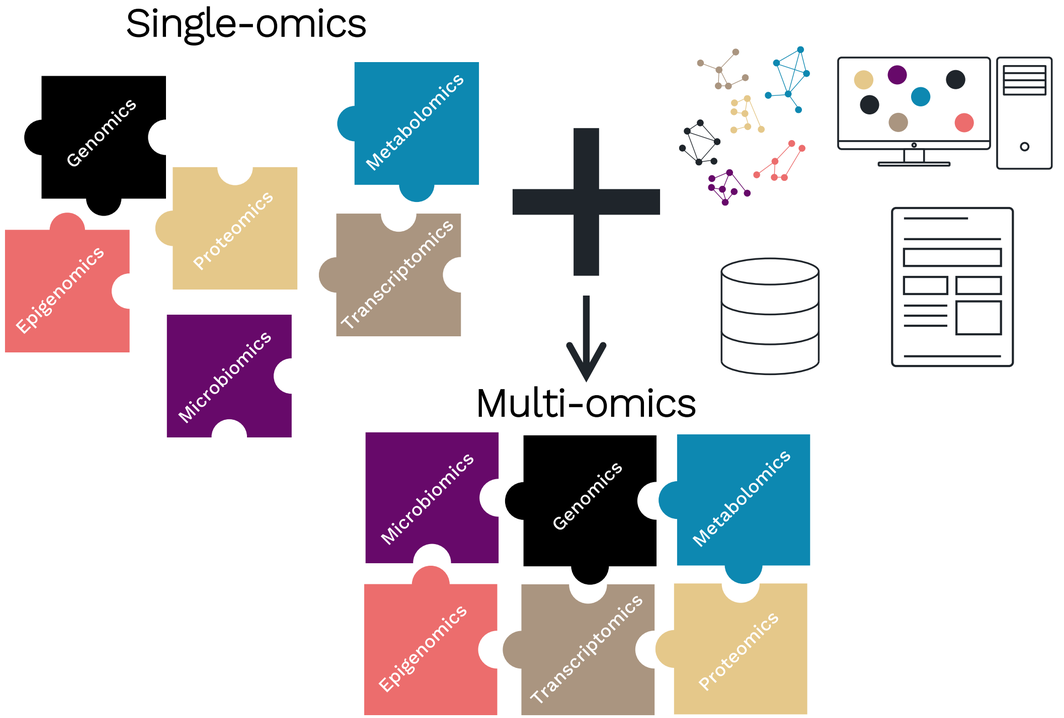


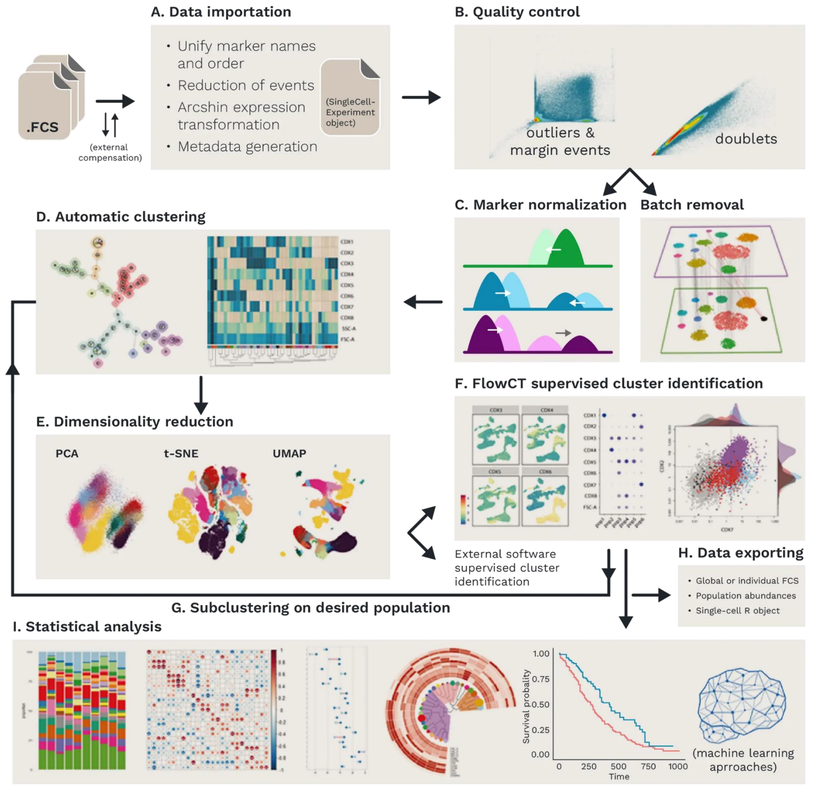
Image adapted from Labory J, et al. ***Front Mol Biosci. 2020;***7:590842.

Latest developments in single-cell technologies

Single-cell technologies for the study of hematological malignancies

FlowCT: computational flow cytometry[1]

- FlowCT is a new computational approach that allows semi-automated analysis of flow cytometry data, which has been traditionally analyzed by manual gating.



CD, cluster of differentiation; CT, cytometry; FCS, flow cytometry standard; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding; UMAP, uniform manifold approximation and projection.

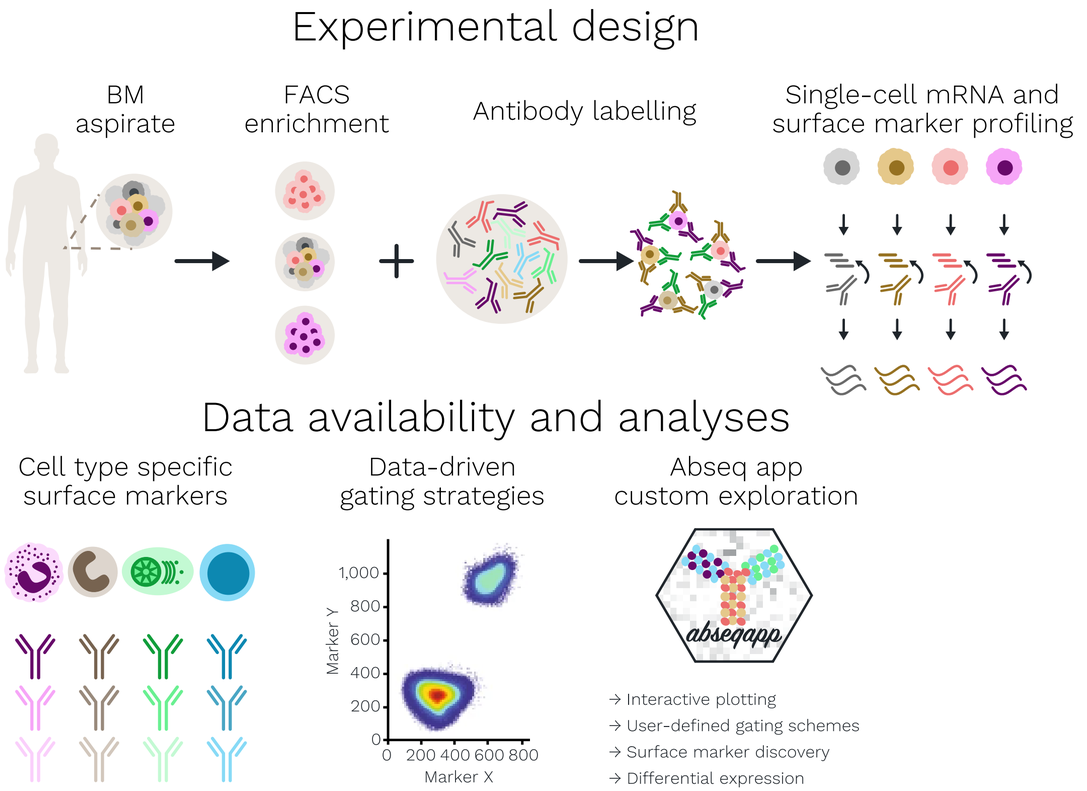
1. Botta C, et al. Blood Adv, 2022;6(2):690–703.
Botta C. The path to single-cell genomics through flow-cytometry (focus on myeloma). Oral presentation at EHA2024.

Latest developments in single-cell technologies

Single-cell technologies for the study of hematological malignancies

CITE-seq/Abseq: the bridge between flow cytometry and –omics[1]

These technologies are based on the use of oligonucleotide-conjugated antibodies to obtain transcriptomic and proteomic information from the same cells, which are stained using traditional flow cytometry staining protocols.



BM, bone marrow; CD, cluster of differentiation; CITE-seq, Cellular Indexing of Transcriptomes and Epitopes by sequencing; FACS, Fluorescence-Activated Cell Sorting; mRNA, messenger RNA.

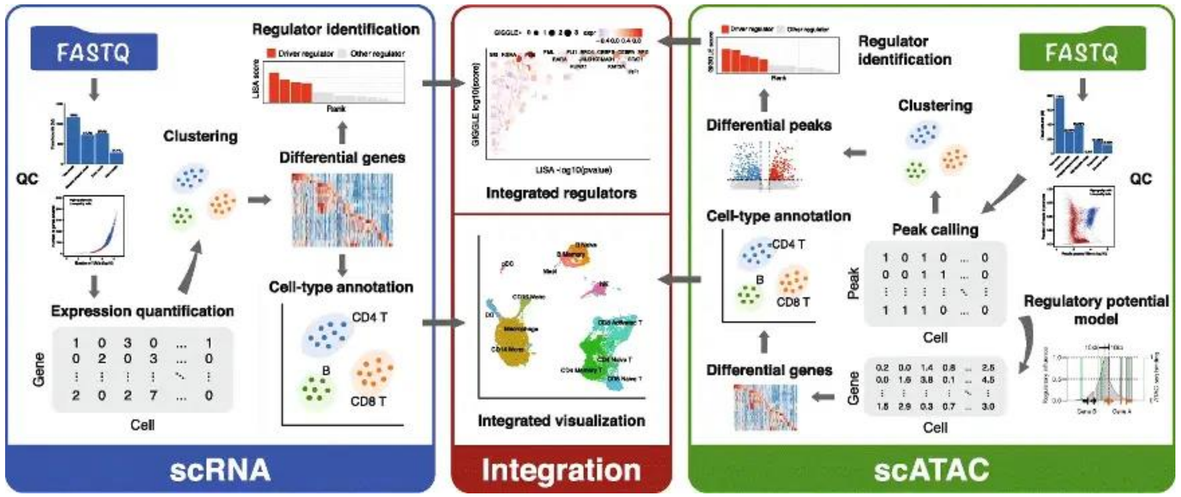
1. Triana S. *Nat Immunol.* 2021;22(12):1577-1589.
Botta C. The path to single-cell genomics through flow-cytometry (focus on myeloma). Oral presentation at EHA2024.

Latest developments in single-cell technologies

Single-cell technologies for the study of hematological malignancies

MAESTRO: Model-based AnalysES of Transcriptome and RegulOme[1]

MAESTRO is a computational workflow for the integrative analysis of scRNA-seq and scATAC-seq, which can provide information on chromatin accessibility and help identify transcriptional regulators of malignancy in specific cell populations.



ATAC, Assay for Transposase-Accessible Chromatin; CD, cluster of differentiation; QC, quality check; sc, single-cell; seq, sequencing.

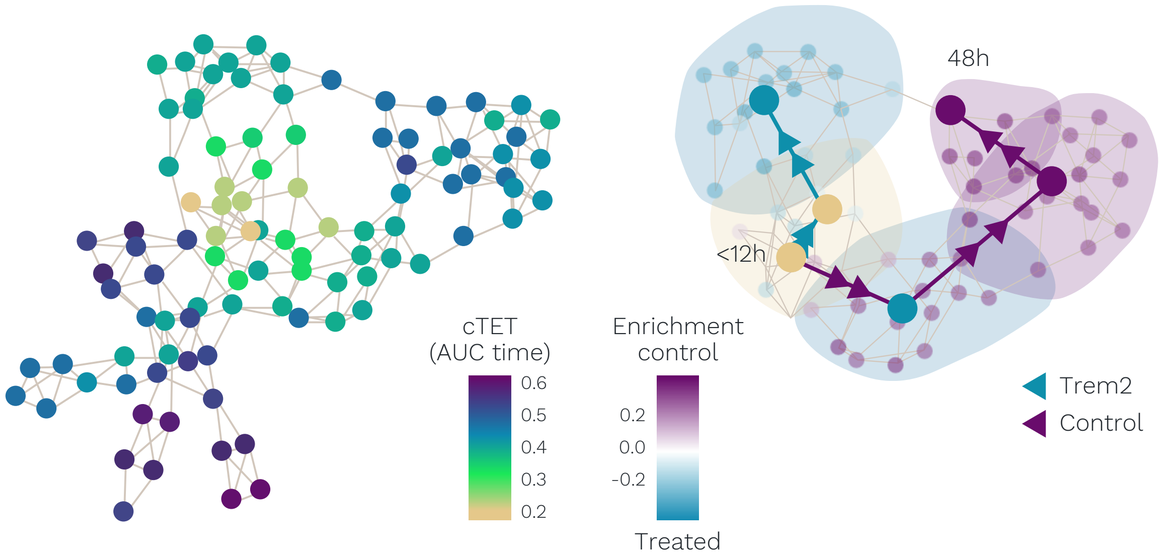
1. Wang C, et al. *Genome Biology.* 2020;21(1):198.
Botta C. The path to single-cell genomics through flow-cytometry (focus on myeloma). Oral presentation at EHA2024.

Latest developments in single-cell technologies

Single-cell technologies for the study of hematological malignancies

Zman-seq: time-resolved single-cell genomics[1]

Zman-seq is a new technology that enables the dynamic measurement of differentiation trajectories across time, which may contribute to the understanding of immune adaptation in response to treatment and enhance the development of more effective immunotherapies.



AUC, area under the ROC curve.

1. Kirschenbaum D, et al. *Cell.* 2024;87(1):149-165.

Amit I. Single cell technologies - Methodological advances for the study of normal and malignant hematopoiesis. Oral presentation at EHA2024.

Latest developments in single-cell technologies

Single-cell technologies for the study of hematological malignancies

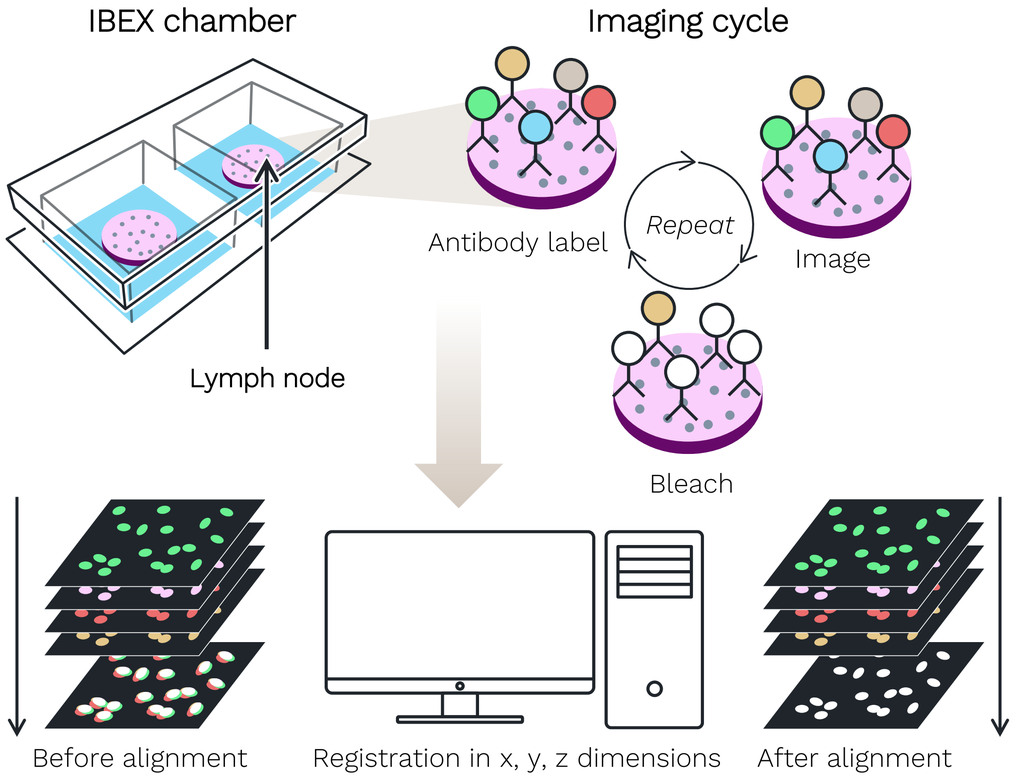
IBEX: Iterative Bleaching Extends multi-pleXity[1]

IBEX is a highly multiplexed imaging method that uses fluorescently-labeled antibodies for spatial proteomic profiling of tissues

- It is versatile and can be used on multiple tissues and sample preparations
- A single tissue section can be incubated with over 60 antibodies at once, which are imaged in cycles with dye inactivation after each cycle
- Individual images can be processed and aligned with open-source software that creates a composite image
- It provides images at high single-cell and spatial resolution, which can help in the study of rare cell populations

IBEX imaging community: open science community with videos to help with the implementation of this technology and a discussion forum where scientists can share their successes and failures (available from: https://ibeximagingcommunity.github.io/ibex_imaging_knowledge_base/)

Single and spatial technologies for analyzing the FL TME were recently reviewed in *Blood*[2]



IBEX, Iterative Bleaching Extends multi-pleXity; FL, follicular lymphoma; TME, tumor microenvironment.

1. Radtke AJ, et al. *Nat Protoc.* 2022; 17(2):378-401. 2. Radtke AJ and Roschewski M. *Blood.* 2024;143(12):1069–1079.

Radtke AJ. Characterization of the follicular lymphoma tumor microenvironment using advanced 2D imaging. Oral presentation at EHA2024.

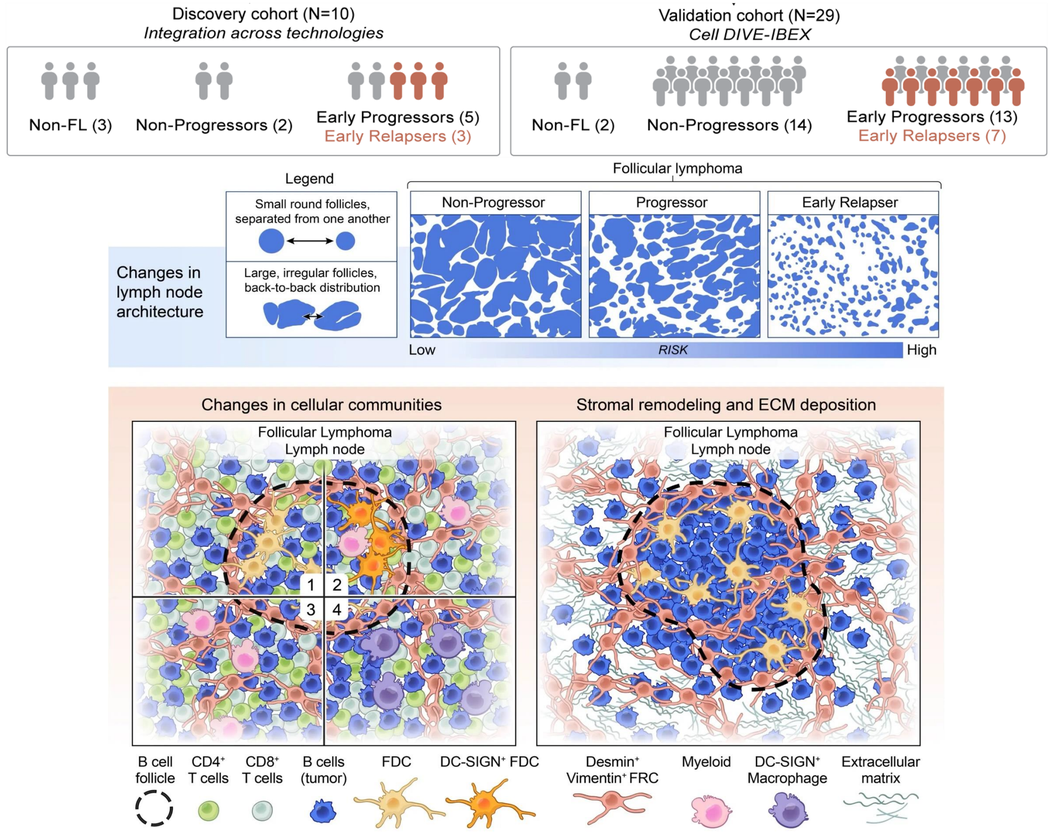
Clinical applications of single-cell technologies

Multi-omic imaging to predict disease outcomes in FL

IBEX imaging of tissue samples from patients with FL[1]

- Excisional biopsies from FL patients were obtained at the time of enrollment and before treatment in a prospective clinical trial
- The aim was to identify tumor B cell-specific features and histological patterns enriched in high-risk patients that could help predict disease behavior and therapeutic outcomes
- Samples from patients in the discovery cohort were analyzed using a combination of four sequencing and imaging technologies for RNA and proteomic profiling, and key spatial findings were then validated using IBEX in a larger cohort
- By integrating clinical and experimental data, this study generated a multi-omic atlas of the follicular lymphoma tumor microenvironment
    - Distinct follicular growth patterns were observed in patients 20 months before relapse
    - Enhanced stromal remodeling and ECM deposition was observed in aggressive clinical cases

Future directions: “static snapshots” do not address tumor evolution between sites and over time → important to collect multi-omic data from different lymph nodes together with longitudinal profiling via liquid biopsies



CD, cluster of differentiation; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; ECM, extracellular matrix; FDC, follicular dendritic cell; FL, follicular lymphoma; FRC, fibroblastic reticular cell; IBEX, Iterative Bleaching Extends multi-pleXity.

1. Radtke AJ, et al. *Cancer Cell*. 2024;42(3):444-463.
Radtke AJ. Characterization of the follicular lymphoma tumor microenvironment using advanced 2D imaging. Oral presentation at EHA2024.

Clinical applications of single-cell technologies

Single-cell genomics to predict response to CAR T treatment in DLBCL

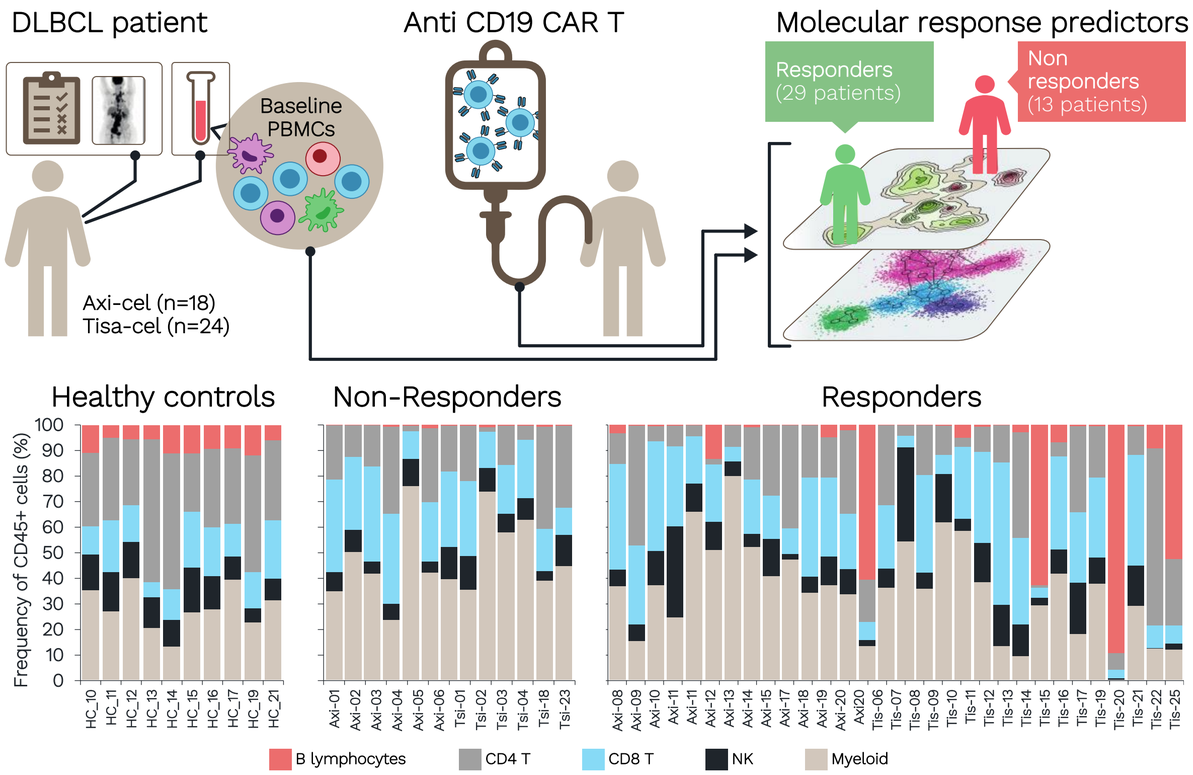
Single-cell genomic analysis of samples from DLBCL patients receiving CAR T treatment

- Surprisingly, pre-treatment PB carried the highest amount of predictive value compared to CAR T bags and CAR Ts post-treatment

Distinct myeloid compositions and molecular states in pre-treatment PB were found to effectively predict responses to CAR T therapy:

- Malignant and healthy-like B cells in PB were associated with favorable responses to CAR T treatment
  - Circulating B cells from DLBCL patients expressed many driver genes and carried CNVs in regions associated with B cell malignancies (e.g.: *BCL2*)
  - Malignant B cells were characterized by an unbalance in κ/λ light chain gene products
- The responder group was also defined by a strong downregulation of myeloid cells
  - Specifically, there was a decrease in classical CD14 monocytes, while non-classical CD16 monocytes were significantly increased in this group

Future directions: single-cell analysis of PB in routine clinical practice to predict outcomes of CAR T therapy and improve patient selection in DLBCL and other malignancies



BCL2, B-cell lymphoma 2; CAR T, chimeric antigen receptor T-cell; CD, cluster of differentiation; CNV, copy number variation; DLBCL, diffuse large B-cell lymphoma; NK, natural killer; PB, peripheral blood; PBMC, peripheral blood mononuclear cells.
Amit I. Single cell technologies - Methodological advances for the study of normal and malignant hematopoiesis. Oral presentation at EHA2024.

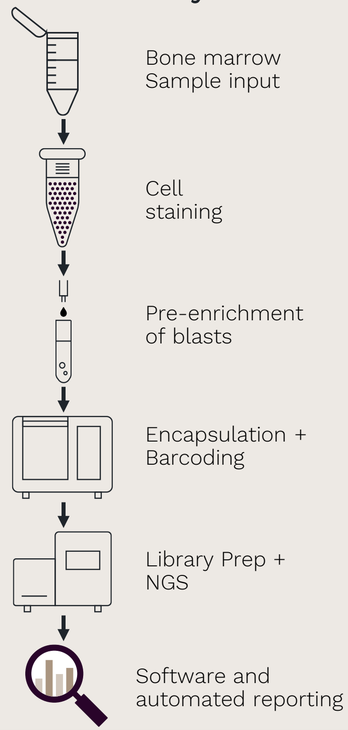
Clinical applications of single-cell technologies

Multi-omics approach for simultaneous detection of SNVs, CNVs, and cell-surface proteins at the single-cell level

scMRD assay for simultaneous DNA mutation and surface immunophenotype profiling

- Discriminating between residual leukemic clones and normal precursors remains a challenge with current tools for MRD assessment
- By integrating genotypic and immunophenotypic assessment in the same individual cells, this multi-omics assay enables single-cell MRD (scMRD) measurement with high sensitivity and specificity
- Single-cell proteogenomics reveals the complexity of disease, including comprehensive insights into clonal architecture, clone-specific acquired mutations, zygosity, genome-wide structural variations, and surface immunophenotypes
- Even if this technology may not be applicable in routine clinical practice yet, understanding the biology that allows MRD clones to resist therapy is necessary to guide the development of more effective and personalized treatments

scMRD assay: workflow



AML, acute myeloid leukemia; CNVs, copy number variation; MRD, minimal residual disease; NGS, next-generation sequencing; scMRD, single-cell MRD, SNVs, single-nucleotide variants.

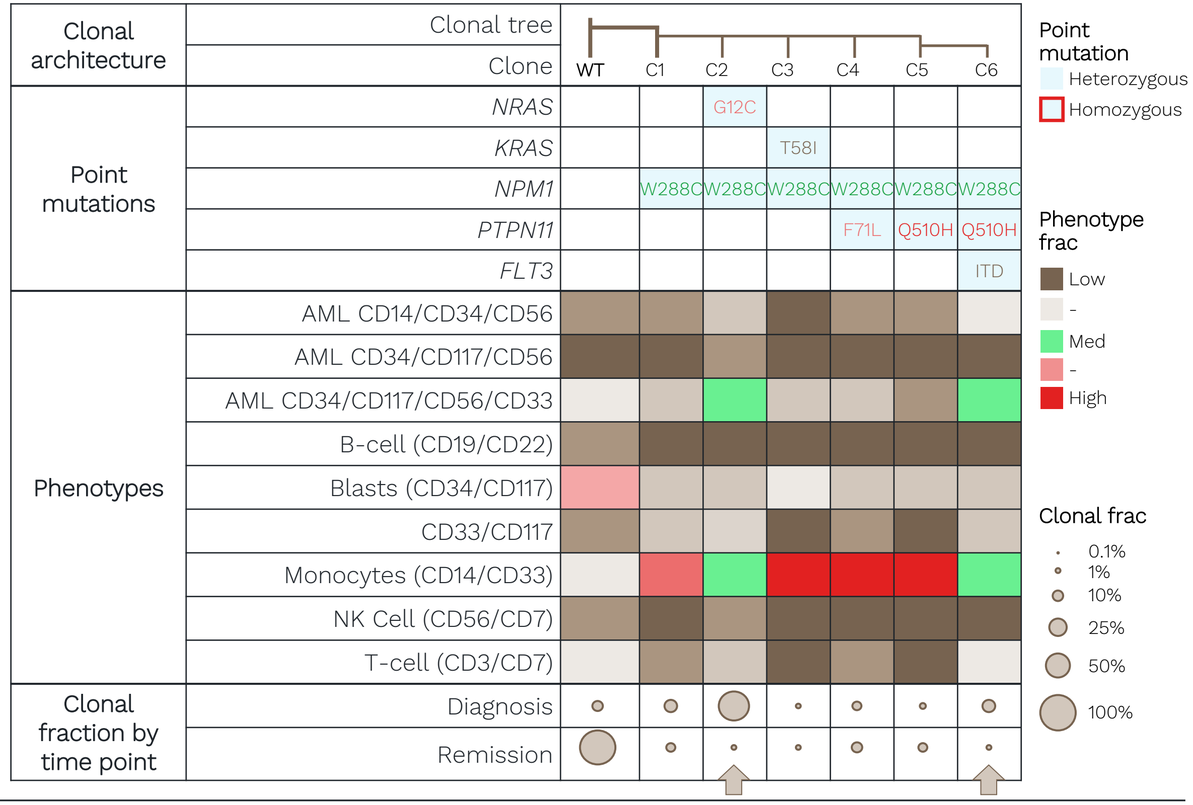
Fuerte G. A novel single-cell measurable residual disease (scMRD) assay for simultaneous DANN mutation and surface immunophenotype profiling. Abstract S337 presented at EHA2024.

Clinical applications of single-cell technologies

Multi-analyte analysis of clones and time points using scMRD assay

Case study

- AML clones were detected in a patient who had negative MRD by flow cytometry
- Despite low tumor burden, the clonal architecture was recreated from an *NPM1* W288 founder mutation
- Other actionable mutations, such as *FLT3*, were detected even if the frequency was low
- Mutant clones were mostly mature monocytes, but the dominant clones at diagnosis had a complex CD34/CD117/CD56/CD33 phenotype



AML, acute myeloid leukemia; CD, cluster of differentiation; MRD, minimal residual disease; NK, natural killer; scMRD, single-cell MRD.

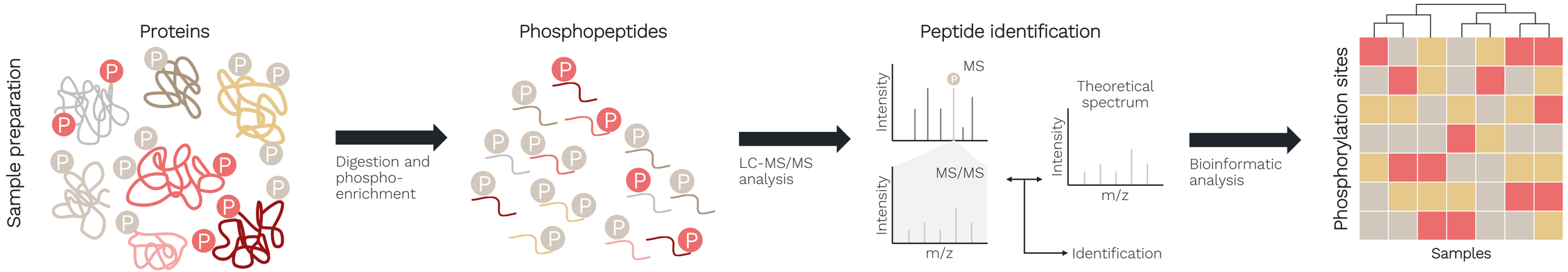
Fuerte G. A novel single-cell measurable residual disease (scMRD) assay for simultaneous DANN mutation and surface immunophenotype profiling. Abstract S337 presented at EHA2024.

Proteomic technologies

(Phospho)proteomics for precision hemato-oncology

- Protein kinases and phosphatases can drive the activation of oncogenic pathways
- Kinase activity is determined not only by activating or inactivating mutations, but also by other molecular events (e.g. epigenetic modifications, post-translational modifications, etc.)
- By measuring phosphorylation sites, and thus kinase activity, phosphoproteomic tools can help us quantify oncogenic signaling in a way that takes into account many molecular events involved
- Many studies have evaluated the correlation between the activation of kinase signaling pathways and responses to targeted therapies

Phosphoproteomics workflow



LC, liquid chromatography; MS, mass spectrometry; m/z, mass to charge ratio; P, phosphorylation.
Cutillas P. Proteomic profiling for precision hematology. Oral presentation at EHA2024.

Proteomic technologies: a clinical application

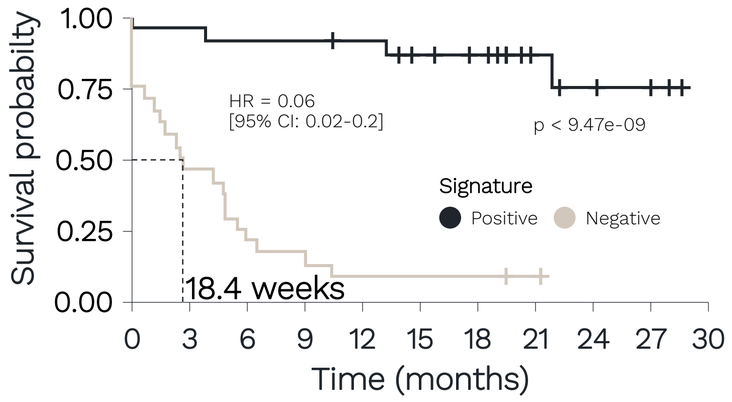
Phosphoproteomics to predict response to M+IC in AML

- Midostaurin was the first kinase inhibitor approved to treat AML in *FLT3*^{mut} patients
- Genetic mutations often fail to predict the best treatment for a given patient
  - >40% of *FLT3*^{mut} patients in the RATIFY phase 3 trial were refractory to midostaurin[1]
- In this retrospective clinical study, 47 *FLT3*^{mut} AML patients treated with midostaurin + chemotherapy were analyzed
- Patients positive for a kinase activity signature, which had been identified in a previous preclinical study[2], responded significantly better to treatment than negative patients
- This study demonstrates that phosphoproteomic signatures can be used to accurately predict resistance to treatments, and may be a useful tool to improve patient stratification

Future directions:

- Move to a patient-centric approach by using machine learning on phosphoproteomics data to rank treatments based on predicted efficacy within each patient (clinical validation is in progress)

ML-derived phosphoproteomics signature accurately predicts response to M+IC



Signature	Number at risk								
●	23	22	21	20	18	13	7	3	0
●	24	11	4	2	2	2	0	0	0

AML, acute myeloid leukemia; HR, hazard ratio; M+IC, midostaurin plus immunochemotherapy; ML, machine learning.

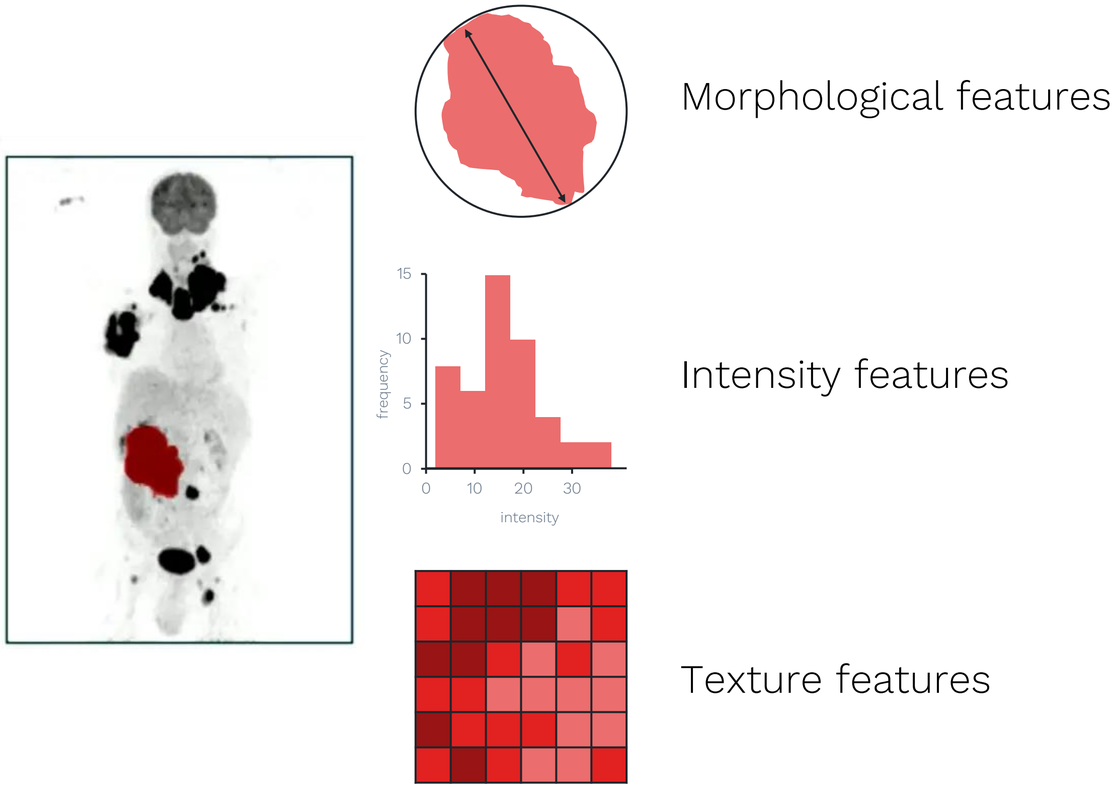
1. Stone RM, et al. N Engl J Med. 2017; 377(5): 454-464. 2. Casado P, et al. Leukemia. 2018; 32(8):1818-1822.
Cutillas P. Proteomic profiling for precision hematology. Oral presentation at EHA2024.

Imaging technologies

PET and radiomics

“Images are more than pictures, they are data”[1]

- PET scans are widely used in routine clinical practice
- Radiomic features, which can be extracted and calculated from PET scans, provide a comprehensive quantification of tumor phenotype
- There are several software available for high-throughput extraction and calculation of radiomics features. E.g.: PyRadiomics, LIFEx
  - These tools can extract up to 500 different features, including morphological, intensity, and texture features
- Radiomics models take into account several radiomics features and can predict disease outcomes



LIFEx, Local Image Features Extraction; PET, positron emission tomography.

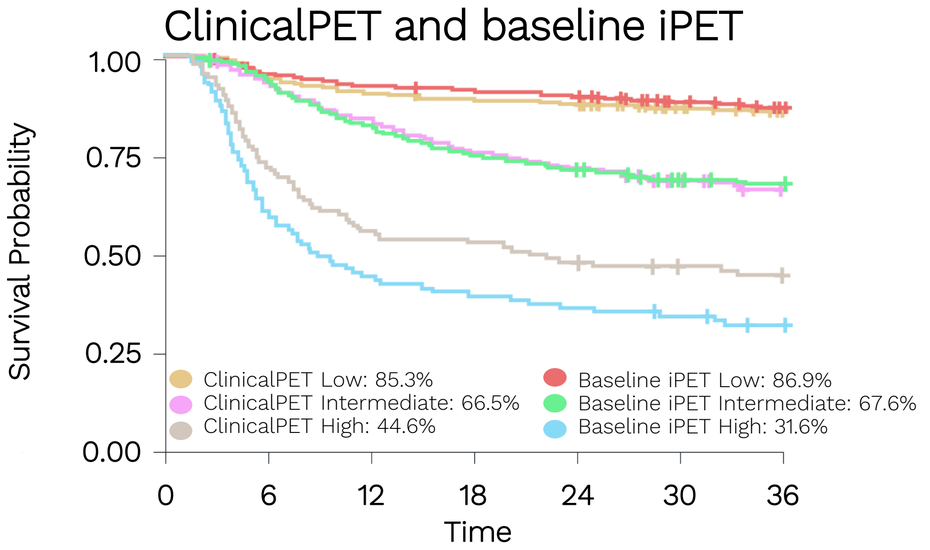
1. Gillies RJ, et al. *Radiology*. 2016;278(2):563-577.
Eertink JJ. The use of PET and radiomics in DLBCL. Oral presentation at EHA2024.

Clinical application of imaging technologies: Use of radiomics models to predict prognosis in DLBCL

- Previous radiomics models were based on baseline radiomics features. ClinicalPET, for example, incorporates MTV, SUV_{peak}, and $Dmax_{bulk}$
- The **baseline iPET model** combines baseline radiomics features with interim PET response and shows significantly improved performance in predicting survival rates of high-risk patients (44.6% with clinicalPET vs. 31.6% with baseline iPET)

Future directions:

- AI-based methods for automatic segmentation of lesions and direct predictions derived from PET scans could help bring radiomics into routine clinical practice



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

AI, artificial intelligence; DLBCL, diffuse large B-cell lymphoma; $Dmax_{bulk}$, maximum distance between the target lesion and another lesion; iPET, interim PET; MTV, metabolic tumor volume; PET, positron emission tomography; SUV_{peak}, peak standardized uptake value.

Eertink JJ. The use of PET and radiomics in DLBCL. Oral presentation at EHA2024.

Conclusion

- Many omics technologies, including single-cell genomics, proteomics, multi-omic imaging and radiomics, have been shown to accurately predict disease outcomes or responses to treatment in a number of hematological malignancies
- Implementing these technologies in routine clinical practice, is a current challenge because of the high costs and complex data analysis
    - Machine learning and AI-based tools may help simplify data analysis and interpret complex results

AI, artificial intelligence.

04

Artificial intelligence & machine learning

AI-based tools & applications in hematology

The revolution of AI and machine learning tools in the field of hematology

- Since the launch of ChatGPT, artificial intelligence (AI) has had a significant impact on our society
- AI promises to increase productivity, boost efficiency, increase insights into big data and transform the life cycle of medicines, healthcare and public health
- AI and machine learning (ML) are often used interchangeably, but ML is a subset of the broader category of AI[1]
- AI is a system that reasons, understands, and acts to solve a complex problem autonomously, whereas ML attempts to computationally extract and predict meaningful outcomes from complex data structures by learning from training data[1]
- AI is expected to significantly impact hematology practice in various areas, such as screening, diagnostics, genomics, and drug testing[2]

An image generated using an AI tool depicting physician-patient interaction in the era of AI tools[3]

1. Shouval R, et al. *Brit J Haemat.* 2021;192(2):239–250.
2. Rösler W, et al. *J Can Res Clin Oncol. 2023;*149(10):7997–8006.
3. OpenAI, "Doctor Conducting a Patient Appointment with AI Assistance," 2024

Potential areas to enhance patient care using AI in hematology

1. Enhancing patient-physician interaction

- Record and transcribe conversations between physicians and patients, allowing physicians to focus more on the patient
- Write medical history reports and plan tests/appointments

2. Automating diagnosis and testing

- Perform tasks such as automated karyotyping and cell classification, traditionally performed by experienced technicians
- Use of Large language model to generate reports

3. Personalizing treatment plans

- Assist in creating treatment plans based on patient data, considering factors such as combination therapy and side effects

4. Research and clinical trials

- Aid in knowledge summarization and automate data analysis, expediting the clinical trial process and the development of new treatments

5. Monitoring patient health and supporting engagement

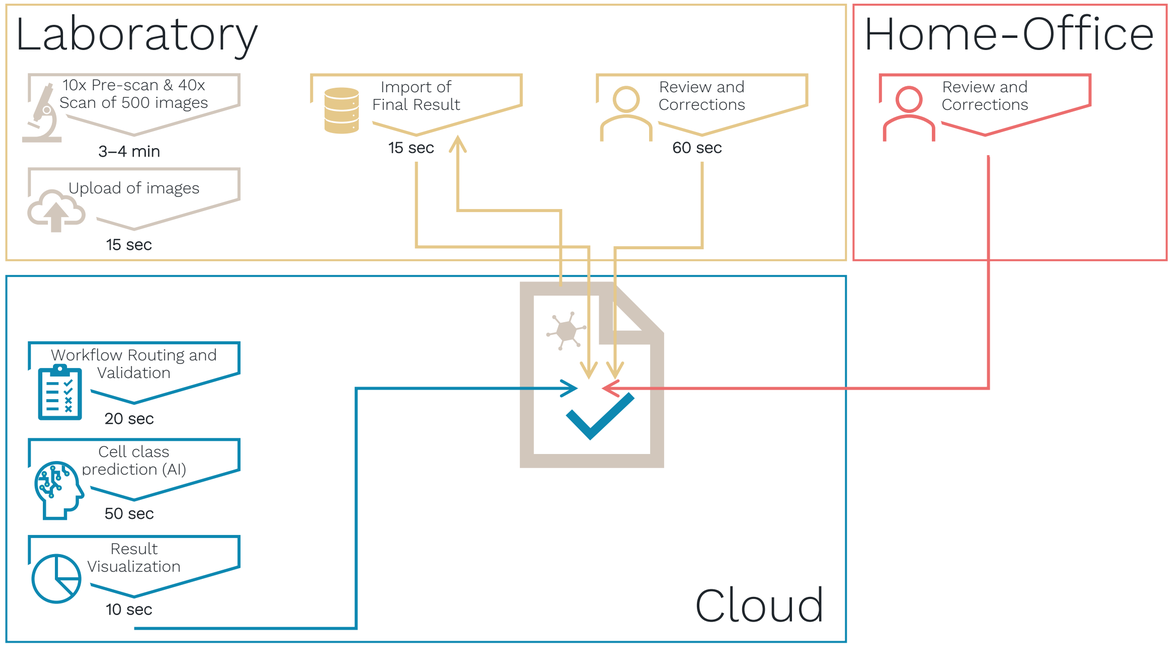
- Use of chatbots to provide patient consultations, potentially serving as a valuable tool in patient engagement

AI, artificial intelligence.

Haferlach T. Artificial intelligence (AI) in clinical hematology practice. Oral presentation at EHA2024.
Haferlach T. AI applicability in the field of hematology. Oral presentation at EHA2024.

AI automation in diagnosis

Machine learning in morphological diagnosis of hematological diseases



BELUGA: Integration of the AI-driven cloud-based platform into routine diagnostic workflow[1]

- Cytomorphology and differential count of peripheral blood are routine methods used in hematology; however, they are often labor-intensive, time-consuming, and not entirely reproducible[1]
- Deep Neural Networks (DNNs) are frequently used in machine learning (ML) for diagnostic purposes and are becoming increasingly superior to human examiners in accuracy and speed
- A prospective, blinded clinical trial (BELUGA*) compared an AI-driven cloud-based platform to conventional manual examination in peripheral blood differential count with >29,000 cases[1]
- The results showed a 94.5% concordance between the two methods, with the AI-driven platform demonstrating higher reproducibility and shorter turnaround times compared to the manual approach
- Haemorasis, a computational method to distinguish peripheral differential count, when integrated with cytomorphological features using ML, was able to distinguish *SF3B1*-mutant MDS from other MDS using cytomorphology and blood counts alone, with high predictive performance[2]

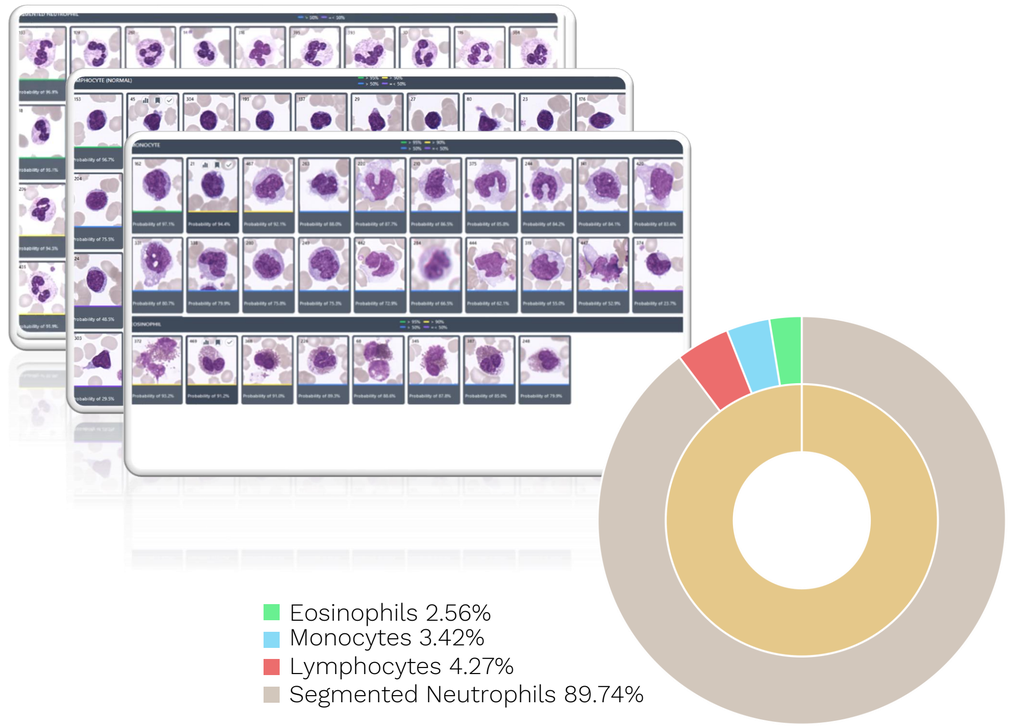
*Clinicaltrials.gov, NCT04466059 https://clinicaltrials.gov/study/NCT04466059.
AI, artificial intelligence; MDS, myelodysplastic syndrome.
1. Haferlach T, et al. *Blood.* 2022;140(Supplement 1):1909–1910. 2. de Almeida JG, et al. *Nature Communications.* 2023;14(1):4378
Haferlach T. Machine learning in morphological diagnosis of hematological disease. Oral presentation at EHA2024.

AI automation in diagnosis

Potential for large-scale incorporation of automated cytomorphology into routine diagnostic workflows

- A multi-step deep learning approach automatically segmented cells from bone marrow images, accurately distinguishing between AML samples and healthy controls, and also predicted the common AML mutation *NPM1* using only image data[1]
- 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[2]
- SCEMILA could perfectly discriminate between AML patients and healthy controls, predict the AML genetic subtypes with high accuracy and identify clinically relevant cells[2]
- A deep learning algorithm for detecting *MYC* rearrangement in scanned histological slides of DLBCL enabled a simple and fast prescreening, leading to approximately 34% reduction in genetic tests[3]
- Collectively, these works revealed the potential to develop a unifying, dynamic model, almost a virtual guide, that integrates different data sources to find the best possible diagnosis for each patient with limited human interference

An example of an AI-based cell classification



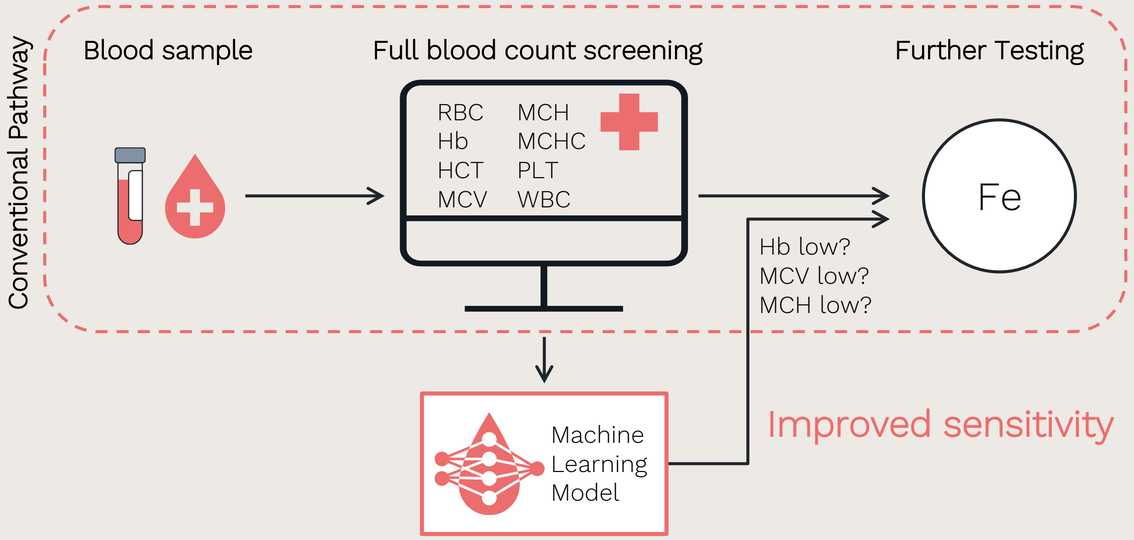
AI, artificial intelligence; AML, acute myeloid leukemia; DLBCL, diffuse large B cell lymphoma; NPM1, nucleophosmin.

1. Eckardt JN, et al. *Leukemia.* 2022;36(1):111–118. 2. Hehr M, et al. *PLOS Digital Health.* 2023;2(3):e0000187. 3. Swiderska-Chadaj Z, et al. *Virchows Arch.* 2021;479(3):617–621.
Haferlach T. Machine learning in morphological diagnosis of hematological disease. Oral presentation at EHA2024.

Need for improved and reliable screening methods for iron deficiency

- Conventional screening tests fail to identify iron deficiency (ID) in many patients, especially medically vulnerable patient groups
- Data from 48,000 blood donors from the INTERVAL trial was used for this analysis[1]
- Part 1 of the analysis included investigating the sensitivity of current ID screening on a larger scale using two ID definitions
- All single low-thresholds for Hb, MCV, and MCH showed low sensitivity and combining them improved sensitivity but still remained under 50%; over half of the ID cases were missed
- Sensitivity for detecting ferritin <15 µg/L using national FBC reference ranges within a healthy population was low
- Novel FBC measurements with machine learning can be used to detect ID at lower costs and with higher sensitivity than current screening

Adapted iron deficiency screening using a modern ML technique trained on novel high-dimensional full blood counts



FBC, full blood count; Fe, iron; Hb, hemoglobin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet count; RBC, red blood cell; WBC, white blood cell.

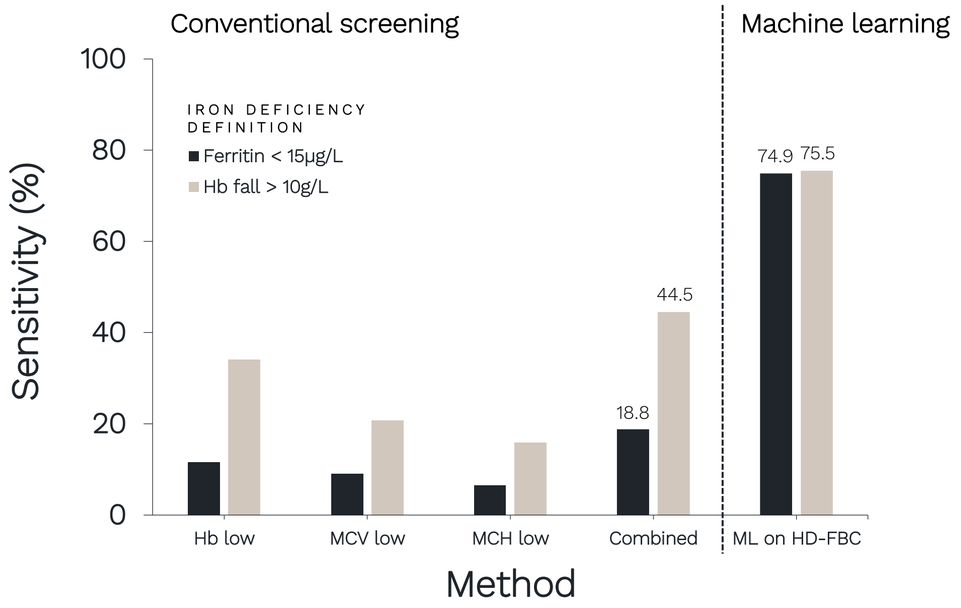
1. Di Angelantonio E, et al. *Lancet*. 2017;390(10110):2360–2371.
Kreuter D. Machine learning to transform iron deficiency screening: from rusty tools to cutting-edge solutions. Abstract S332 presented at EHA2024.

AI automation in screening/testing

Machine learning improved iron deficiency screening efficiency in conventional blood sample testing

- 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
- Work was ongoing to validate the ML model in a second cohort with an ethnically diverse donor population, enhancing the sensitivity of the model and performing a similar analysis in already secured patient data of ~2.4 million patients at partner institutions

Difference in detecting ID between conventional and ML model methods

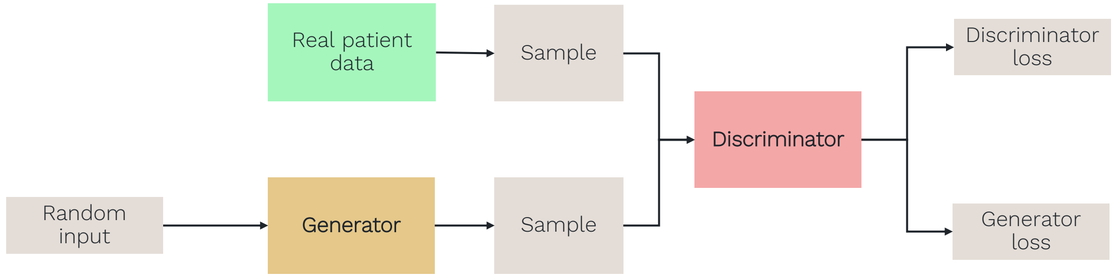


FBC, full blood count; ID, iron deficiency; Hb, hemoglobin; HD, high dimensional; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; ML, machine learning.

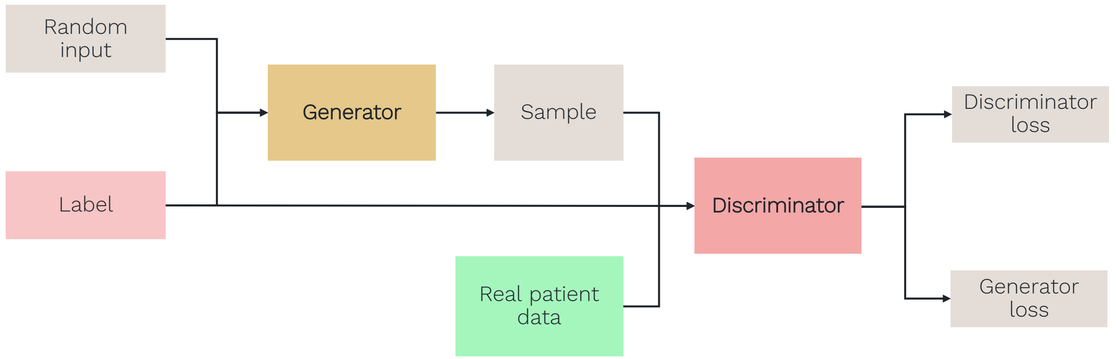
Kreuter D. Machine learning to transform iron deficiency screening: from rusty tools to cutting-edge solutions. Abstract S332 presented at EHA2024.

Creating a synthetic patient dataset using generative AI

Generative Adversarial Networks (GANs)



Conditional GAN architecture



- Collecting patient information for generating clinical evidence in hematology can be challenging, especially for rare and heterogeneous diseases, while privacy issues may restrict data use beyond specific contexts[1]
- Synthetic data can help overcome many of the drawbacks of real 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 data
- Generative Adversarial Networks (GANs), a deep learning-based method, generate artificial outputs, which are then passed to the discriminator along with real data to identify which outputs are real and which are fake
- The presented abstract used a conditional GAN that ensures more precise generation and discrimination modeling of large data sets with complex distribution and interactions among different features
- A synthetic validation framework (SVF) was also developed to evaluate the fidelity and privacy preservability of the newly generated synthetic data

AI, artificial intelligence.

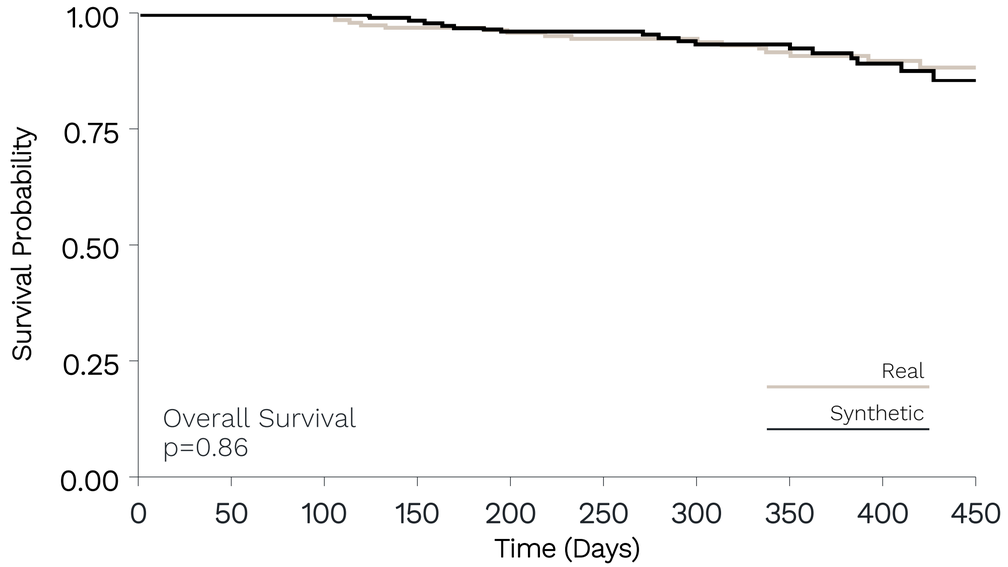
1. D'Amico S, et al. *JCO Clin Can Inform.* 2023;7:e2300021.

Della Porta M. Synthetic patient sets and their potential role in clinical trial acceleration and clinical decision making. Oral presentation at EHA2024.

Synthetic data to accelerate research and precision medicine in hematology

- A cohort of real myelodysplastic syndrome patients from GenoMed4All was used to generate and validate the synthetic data in different experimental settings[1]
- 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[2]
- This technology may increase the scientific use and value of real data and accelerate precision medicine in hematology
- Generative AI can ensure high privacy preservation of newly generated synthetic data

Comparison of clinical trial endpoints between real and synthetic patients



Clinical Endpoint	Real data	Synthetic data	P-value
RBC-TI ≥ 8 weeks 1-24	56 (31.5)	56 (31.5)	1.0
Longest transfusion independece period, weeks, median (range)	195 (56-490)	280 (56-490)	<0.05
RBC-TI ≥ 8 weeks 1-48	68 (38.2)	61 (34.3)	0.50
RBC-TI ≥ 12 weeks 1-24	36 (20.2)	41 (23.0)	0.60
RBC-TI ≥ 12 weeks 1-48	51 (28.7)	46 (25.8)	0.63
Reduction ≥ 4 RBC	62 (34.8)	63 (35.4)	1.0
Reduction ≥ 50%	77 (43.3)	72 (40.4)	0.66

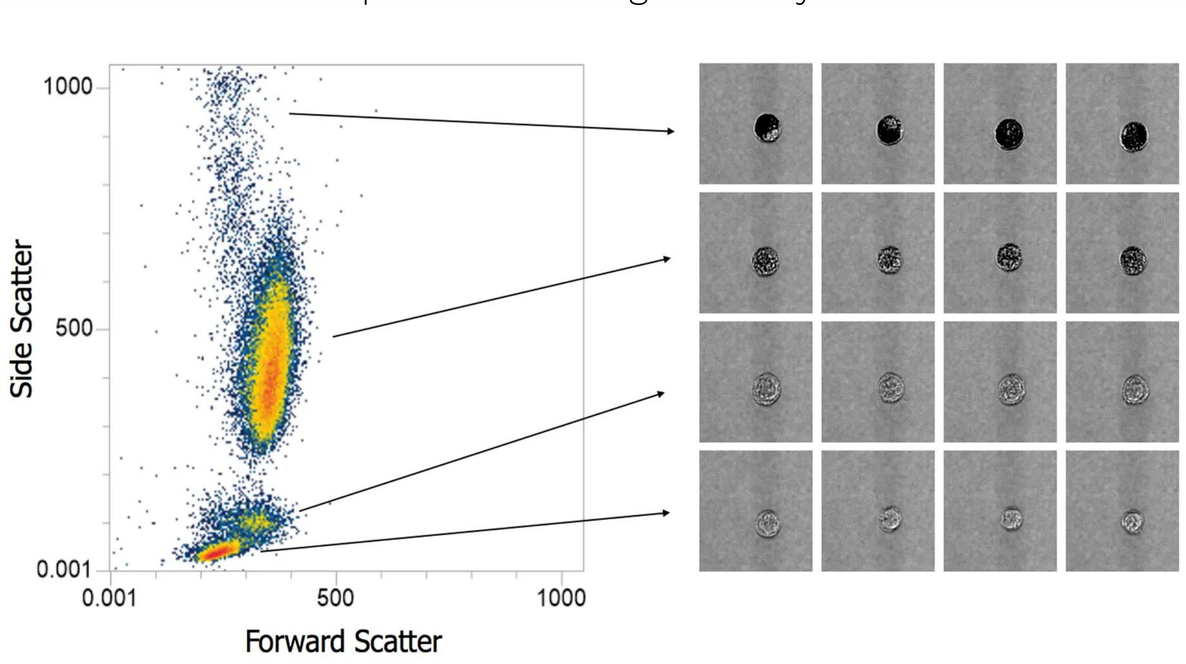
AI, artificial intelligence; GAN, generative adversarial networks; RBC-TI, rate of red blood cell transfusion independence.

1. D'Amico S, et al. *JCO Clin Can Inform.* 2023;7:e2300021. 2. Carrigan G, et al. *Cur Epidemiol Rep.* 2022;9.4:326-337.
Della Porta M. Synthetic patient sets and their potential role in clinical trial acceleration and clinical decision making. Oral presentation at EHA2024.

AI in research

Flow imaging: Combination of flow cytometry with microscope imaging

An example of flow imaging technology used to separate and image leukocytes



- 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
- Multiple configurations are possible with a modular design for 2 to 4 laser systems, up to 14-color flow cytometry and available with violet 6-channel configuration
- It is able to capture up to 6000 images per second with the ability to select which population to image, size measurement tool using images

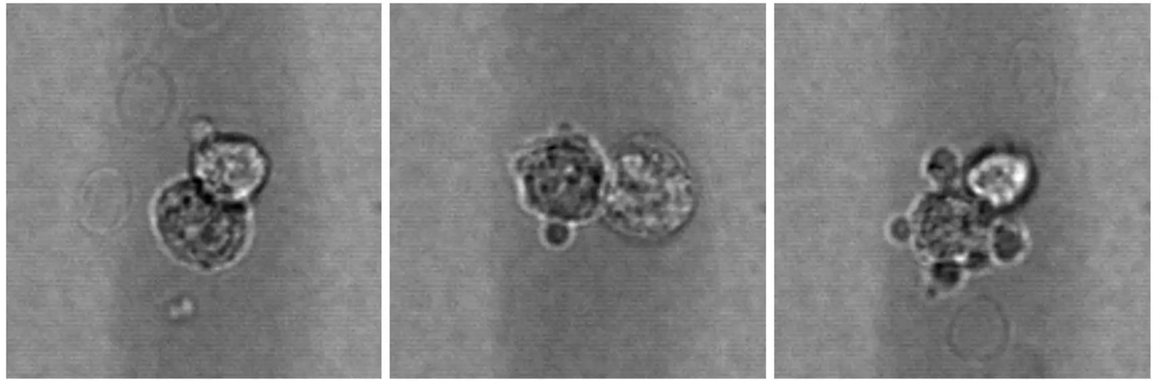
Petriz J. Exploring the frontiers of hematology with flow imaging. Oral presentation at EHA2024.

AI in research

Flow imaging: Versatile research applications with AI-powered software option

- Ability to automatically analyze bright-field images to improve gating strategies and identify rare cells
- Collect brightfield images with and without fluorescent labels
- Particularly suitable for studying cell morphology, rare cell populations, label-sensitive cells, cell-to-cell interactions (cytotoxic activity), apoptosis, DNA content, Ki-67 expression, and other morphological parameters (e.g., presence of Dutcher bodies).
- This versatile and powerful technology is currently for research use only and is not ready for routine clinical practice or diagnostic procedures yet

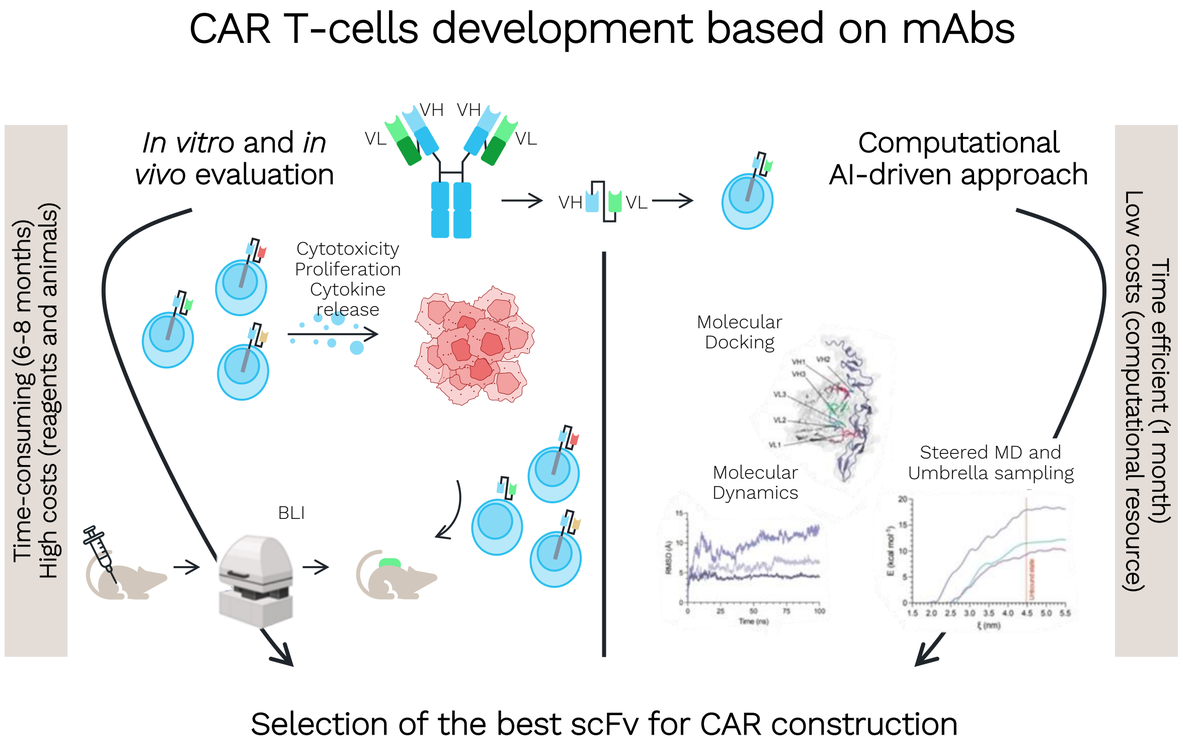
Research applications of flow imaging, such as studying cell-to-cell interactions, specifically cytotoxic activity



AI, artificial intelligence.
Petriz J. Exploring the frontiers of hematology with flow imaging. Oral presentation at EHA2024.

AI in research

Use of AI to advance Chimeric Antigen Receptor design



- AI tools like AlphaFold have sparked a data-driven revolution in biology and medicine by accurately predicting 3D protein structures.[1]
- Selection of mAb-derived scFv is a crucial step in CAR construction to ensure accurate and effective CAR 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
- In the presented study, the researchers utilized AI tools to investigate the molecular antigen-antibody interactions of three different anti-CD30 mAbs for the development of CD30 CAR T-cells against Hodgkin's lymphoma[2]
- AlphaFold2, a deep learning-based method, predicted the 3D structure of both antibody clones and CD30 antigens
- Molecular Dynamics (MD) simulations were then performed to identify the most stable complex and calculate the energy required to dissociate the antibody-antigen binding

3D, 3-dimensional; AI, artificial intelligence; BLI, bioluminescent imaging; CAR, chimeric antigen receptor; mAbs, monoclonal antibodies; scFv, single chain variable fragment.

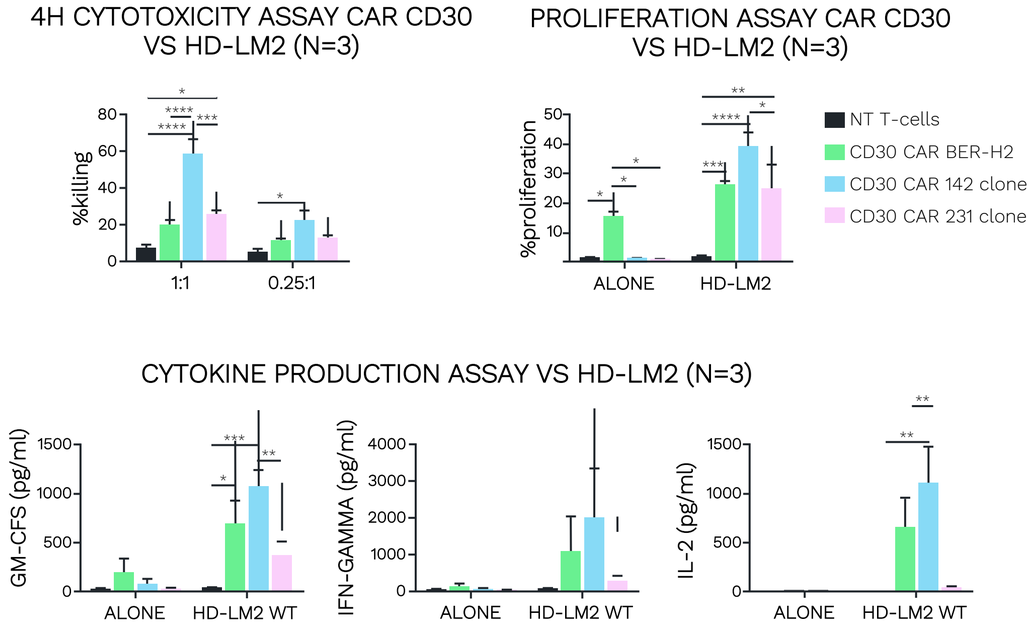
1. Thornton JM, et al. *Nat Med.* 2021;27:1666–1669.
Martarelli N. Artificial intelligence-powered molecular docking for proper scFv selection of anti-CD30 chimeric antigen receptor (CAR). Abstract S280 presented at EHA2024.

AI in research

Virtual scFv selection showed comparable results to SPR and functional assays

- 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 SPR
- 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 vitro evaluation of anti-CD30 CAR T-cells derived from analyzed scFv



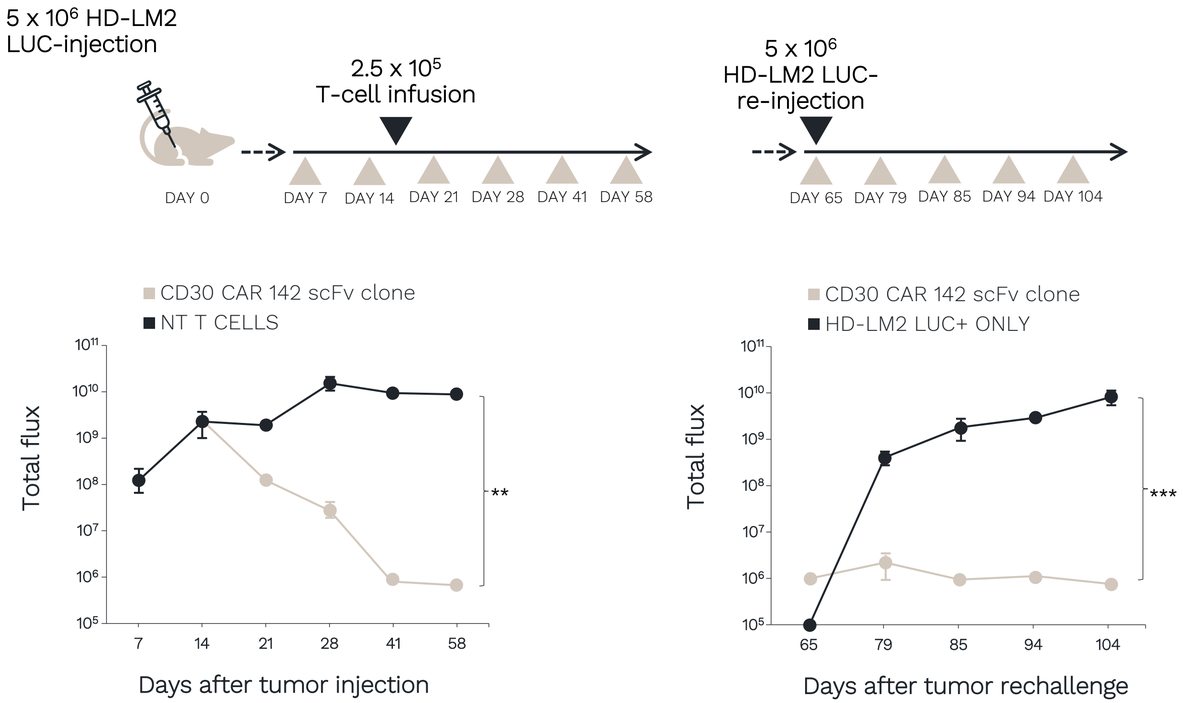
CAR, chimeric antigen receptor; mAbs, monoclonal antibodies; scFv, single chain variable fragment.

Martarelli N. Artificial intelligence-powered molecular docking for proper scFv selection of anti-CD30 chimeric antigen receptor (CAR). Abstract S280 presented at EHA2024.

AI in research

Potential to advance the development of novel CAR constructs

In vivo evaluation of CD30 CAR 142 scFv clone



- *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 highlight the potential to streamline the selection of scFv from mAbs and advance the development of CAR constructs.
- This 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

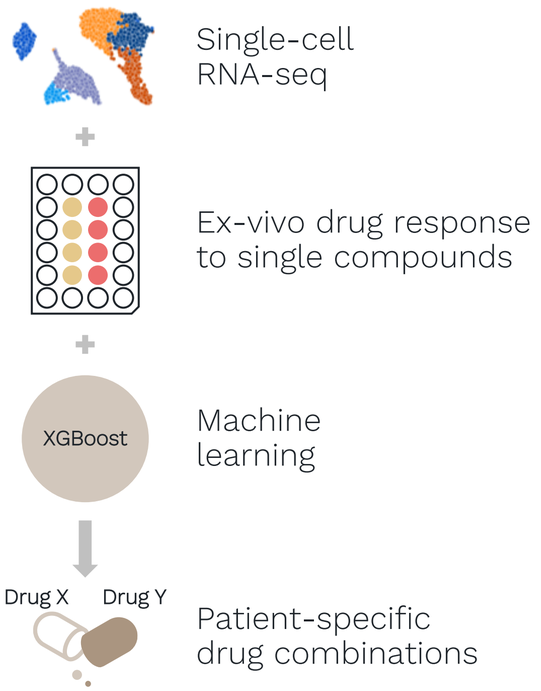
CAR, chimeric antigen receptor; mAbs, monoclonal antibodies; scFv, single chain variable fragment.

Martarelli N. Artificial intelligence-powered molecular docking for proper scFv selection of anti-CD30 chimeric antigen receptor (CAR). Abstract S280 presented at EHA2024.

Use of machine learning for personalized identification of multi-targeting treatments

- Advanced cancers exhibit intratumoral heterogeneity, necessitating personalized and possibly combination therapies to enhance patient outcomes
- However, it is challenging to identify patient-specific treatments due to the vast number of possible drug-dose combinations and the scarcity of patient cells for testing
- Previously, an effective machine learning (ML) approach based on XGBoost that prioritized patient-customized drug combinations with a desired synergy-efficacy-toxicity balance by combining single-cell RNA sequencing with *ex vivo* single-agent testing in scarce patient-derived primary cells was tested[1]
- Major flaws of current machine learning methods: not targeting cancer subclones; lack of preclinical toxicity predictions; dose-specific prediction of responses

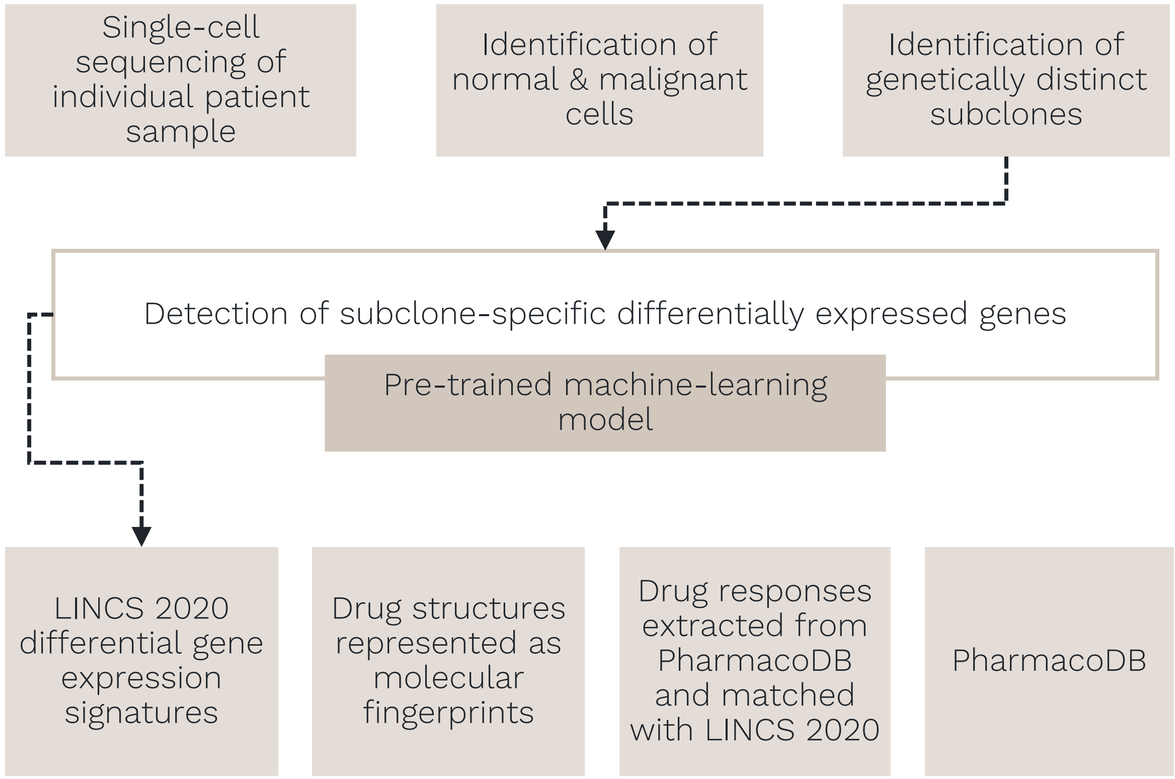
ML algorithm to tailor drug combination for individual patients



1. Ianevski, A., et al. *Sci Adv.* 2021;7(8)eabe4038.
Aittokallio T. Predicting combination therapies. Oral presentation at EHA2024.

AI in personalizing treatment

scTherapy: A novel experimental-computational approach for testing drug combination therapies



Experimental-computational prediction approach

- scTherapy, a machine learning model, was presented that uses scRNA-sequencing data to identify cancer-selective and low-toxic multi-targeting options for individual cancer patients[1,2]
- Predictions are based on transcriptomic differences between genetically distinct cancer cell populations in individual patient samples
- By pretraining 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 drug perturbations, a ranked list is generated of the most effective multi-targeting options for a given patient
- This makes functional ex vivo drug testing more feasible by prioritizing the most potent multi-targeting options for further experimental validation in scarce patient cells
- Future studies should aim to tailor input data to be patient-specific and drug-class-specific by considering differences in binding affinities, phenotypic profiles, and treatment time points

LINCS, Library of Integrated Network-Based Cellular Signatures.

1. Ianevski, A., et al. *BioRxiv*. 2023.06.26.546571.

Aittokallio T. Predicting combination therapies. Oral presentation at EHA2024.

Conclusion

- Artificial Intelligence (AI) is here to stay. AI tools will dramatically change all aspects of hematology, from prevention to diagnosis to targeted treatment
- AI has an immediate impact and assists in the better and personalized management of individual patients
- It can 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
- AI can help healthcare professionals perform their tasks more effectively and efficiently, ultimately leading to improved patient care
- There is a responsibility to test, challenge, and enhance AI models to advance healthcare, improve clinical outcomes and extend patients' lives.

05

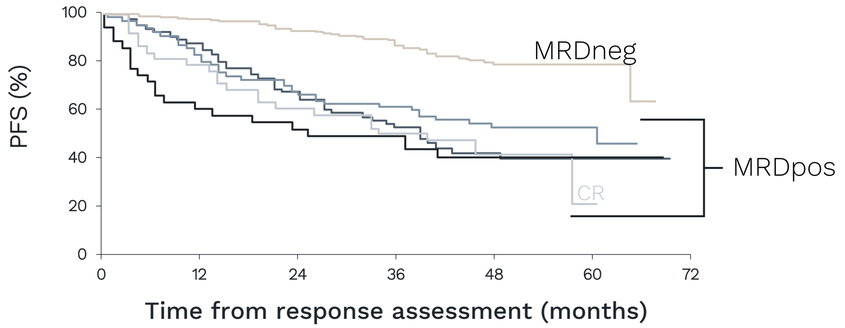
New tools for diagnosis & testing

Recent and future tools for response assessment in multiple myeloma

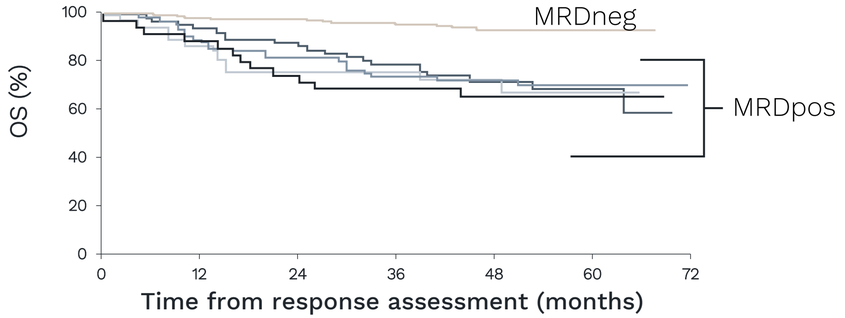
Introduction

- Clinical outcomes in multiple myeloma are still based on conventional response criteria (PFS, ORR, OS)
  - Future criteria and surrogate markers are necessary to implement sustained MRD negativity as “the new CR in multiple myeloma,” as it is strongly associated with better outcomes in all patient subgroups[1,2]
- MRD assessment is more sensitive and has greater prognostic value than IF and CR as response criteria[3]
- Clinical trials should assess MRD in patients with ≥VGPR to ensure that achieved MRD negativity is captured regardless of persistent M-protein[4]
- Using complementary methods is important, e.g. to detect extramedullary disease via PET/CT in MRD negative patients[5]
- MRD is an informative tool in clinical trials but not a perfect biomarker
- MRD assessment is feasible via NGS, NGF, and/or PET/CT
  → Powerful monitoring tool endorsed by IMWG MRD guideline[6]
  (MS to follow soon)

Progression-free Survival (PFS) by MRD status[2]



Overall Survival (OS) by MRD status[2]



CI, confidence interval; CR, complete response; HR, hazard ratio; IF, immunofixation; IMWG, International Myeloma Working Group; MRD, minimal residual disease; MS, mass spectrometry; NGF, next-generation flow; NGS, next-generation sequencing; ORR, overall response rate; OS, overall survival; PET/CT, positron emission tomography/computed tomography; PFS, progression-free survival; VGPR, very good partial response.

1. Munshi NC et al. *Blood Adv.* 2020;4(23):5988-5999. 2. Jimenez-Ubieto A et al. *Blood.* 2021;138(19):1901-1905. 3. Puig N et al. *Haematologica.* 2024 in press. 4. Paiva B et al. *Blood.* 2022;140(23):2423-2428. 5. Paiva B et al. *Blood Cancer Discov.* 2023;4(5):365-373. 6. Kumar S et al. *Lancet Oncol.* 2016;17(8):e328-e346.
Corre J. Future criteria of responses. Oral presentation at EHA2024. & Paiva B. Innovative tools for disease monitoring in multiple myeloma (incl. next generation MRD / tumor cell analyses). Oral presentation at EHA2024.

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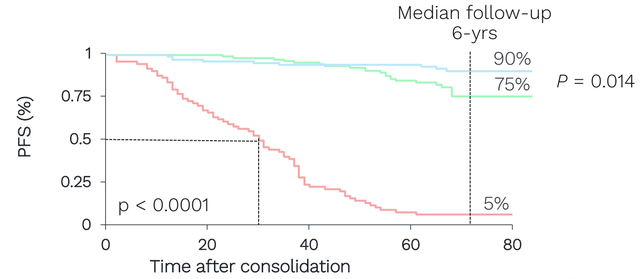
Clinical trials and routine practice

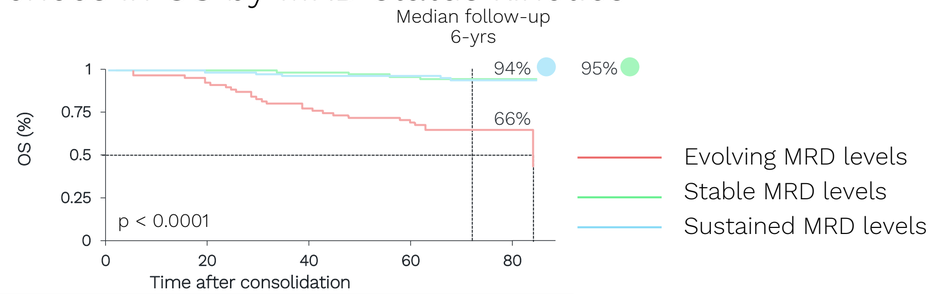
- FDA ODAC hearing committee (04/2024) voted to use MRD negative CR as an early endpoint in clinical trials for accelerated drug approval in multiple myeloma
- Bone marrow MRD assessment is feasible but standardized methods and high-quality samples are necessary
- To use MRD as a clinical outcome parameter, it needs to be assessed periodically as it is dynamic
- MRD assessment is feasible during clinical trials but difficult to achieve in routine practice
  → new, sensitive methods, e.g., blood sampling, are needed
- Blood samples are easier to draw and less invasive, supporting the periodic assessment approach + the use of peripheral blood samples can help to detect new subgroups of patients
- MRD assessment from PB complemented by PET/CT during maintenance/observation phases can reduce the need for BM aspirations (only during induction/intensification)

Differences in PFS by MRD status kinetics[1]



Number at risk					
Evolving MRD levels	73	49	17	5	2
Stable MRD levels	104	104	99	84	5
Sustained MRD levels	100	96	94	90	14

Differences in OS by MRD status kinetics[1]



Number at risk					
Evolving MRD levels	73	68	57	50	9
Stable MRD levels	104	104	103	96	7
Sustained MRD levels	100	99	97	93	14

BM, bone marrow; CR, complete response; EMD, extramedullary disease; FDA, U.S. Food and Drug Administration; LOD, limit of detection; MRD, minimal residual disease; ODAC, Oncologic Drugs Advisory Committee; OS, overall survival; PB, peripheral blood; PET/CT, positron emission tomography/computed tomography; PFS, progression-free survival; yr, year.

Image adapted from Guerrero C et al. *Blood.* 2023;142(Supplement 1):871.

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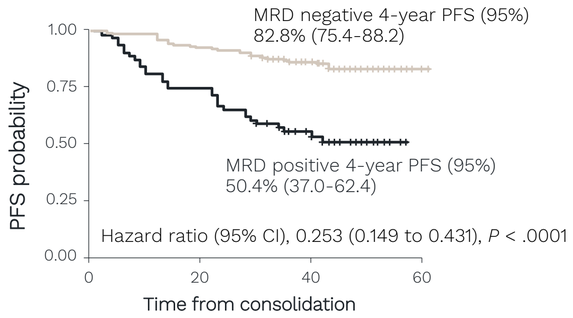
Sustained MRD negativity as marker for treatment interruption?

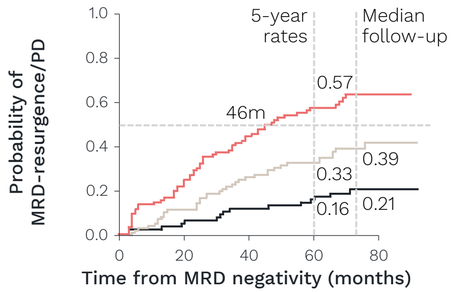
- Results from the GEM2014MAIN trial support the idea of stopping maintenance treatment in patients with sustained MRD negativity on the assumption that MRD is a powerful prognostic factor[1]
- However, there are patients at risk for disease progression / unsustained MRD after treatment is stopped based on MRD negativity
- Therefore, predictors for unsustained MRD are necessary as well
  - Patients who meet the following criteria may not achieve sustained MRD and therefore, treatment should not be stopped:
    (1) ≥2 high-risk cytogenetic abnormalities
    (2) ISS 3
    (3) >0.01% CTCs at diagnosis
    (4) late achievement of MRD negativity[2,3]

Long term PFS by MRD status kinetics[1]



Number at risk							
Rd	63	51	42	31	15	0	
IRd	163	161	149	126	42	4	0

Probability of PD by risk factors[2]



Number at risk					
None	69	65	60	57	28
One	92	81	66	54	15
Two or more	95	74	52	37	8

Risk factors:
One vs. none; HR **2.24**, 95% CI 1.2-4.1, *P*=.008
Two + vs. none; HR **4.39**, 95% CI 2.5-7.7, *P*<0.0001
Two + vs. one; HR **1.96**, 95% CI 1.3-2.9, *P*=.001

ISS	CTCs	Achievement of MRDneg
1 or 2	< 0.01	post-induction (≤6m)
1 or 2	< 0.01	**later (>6m)**
1 or 2	**≥0.01**	post-induction (≤6m)
3	< 0.01	post-induction (≤6m)
3	**≥0.01**	post-induction (≤6m)
3	< 0.01	**later (>6m)**
1 or 2	**≥0.01**	**later (>6m)**
3	**≥0.01**	**later (>6m)**

CI, confidence interval; CTC, circulating tumor cell; HR, hazard ratio; IRd, lenalidomide + dexamethasone + ixazomib; ISS, International Staging System; m, months; MRD, minimal residual disease; MRDneg, MRD negativity; PD, progressive disease; PFS, progression-free survival; Rd, lenalidomide + dexamethasone.

1. Rosiñol L et al. Blood 2023;142(18):1518-1528. 2. Guerrero C et al. Blood 2024;143(7):597-603. 3. D'Agostino M et al. Blood 2024;143(7):592-596.
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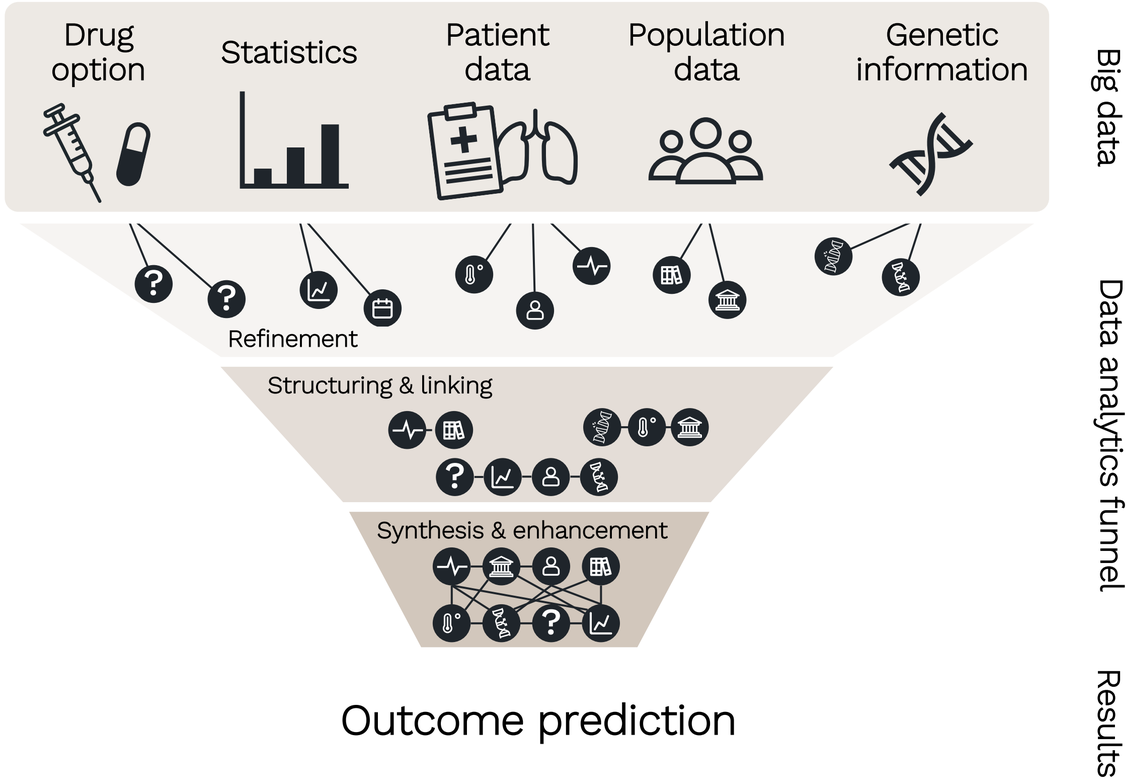
Using big data in MRD assessment and disease development

- Several big data approaches will gain importance in outcome prediction in multiple myeloma
- www.MRDpredictor.com can predict MRD outcomes at diagnosis based on genetic, tumor, and immune biomarkers as an alternative to PFS predictions and, therefore, might help to select the optimal treatment approach for each patient[1]
- The open access tool www.mgus-like.com can help to predict an MGUS-like phenotype at diagnosis via flow cytometry
  → an MGUS-like phenotype is associated with prolonged survival regardless of CR and MRD status[2]
- An open access tool for immune status and MRD monitoring via flow cytometry is in development and could be used to predict the risk for severe infections[3]



CR, complete response; MGUS, monoclonal gammopathy of undetermined significance; MRD, minimal residual disease; PFS, progression-free survival.

1. Guerrero C et al. Clin Cancer Res. 2022;28(12):2598-2609. 2. Burgos L et al. J Clin Oncol. 2023;41(16):3019-3031. 3. Zabaleta A et al. Manuscript in preparation.
Corre J. Future criteria of responses. Oral presentation at EHA2024. & Paiva B. Innovative tools for disease monitoring in multiple myeloma (incl. next generation MRD / tumor cell analyses). Oral presentation at EHA2024.

Conclusion

- MRD status should be considered as an early endpoint for clinical trials in MM with sustained MRD as treatment target
- While bone marrow aspirate is the gold standard for MRD assessment, other, more practical approaches (assessment via blood by NGF/NGS/MS] or via PET/CT), are relevant and might be able to reveal new patient subpopulations (e.g., PRD)
- However, there is no all-encompassing predictor/biomarker and not every result will translate into the same clinical outcome. Therefore, disease monitoring, using tools that are feasible in daily practice, sensitive, and reproduceable is key
- Recommendations and guidelines for these novel tools are needed
- Big data tools can help to predict outcomes in multiple myeloma

MM, multiple myeloma; MRD, minimal residual disease; NGF, next-generation flow; NGS, next-generation sequencing; PET/CT, positron emission tomography/computed tomography; PRD, peripheral residual disease.
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Ultrasensitive biosensor for non-invasive diagnosis of T-lymphoblastic leukemia/lymphoma

Introduction

- T-ALL/T-LBL are highly malignant, rapidly progressing neoplasms with poor prognosis. They are difficult to diagnose early and monitor
- The traditional diagnosis is based on histopathology and immunoassays; however, both have limitations
- Liquid biopsy approaches are an alternative, but these are also limited due to low target content, high cost, low sensitivity and poor specificity of drug-resistant mutations
  → To overcome these constraints, new, accurate, affordable, specific, and fast molecular diagnostic tools are needed
- The CRISPR/Cas system offers its specific recognition ability and high efficiency → CRISPR/Cas-based diagnostics[1]
- However, CRISPR/Cas-based diagnosis lacks applications for multiplex detection and the reaction requires multiple steps
- An affordable, sensitive, specific, rapid, equipment-free diagnostic CRISPR biosensor for T-ALL/T-LBL should be established in an one-step/one-pot approach

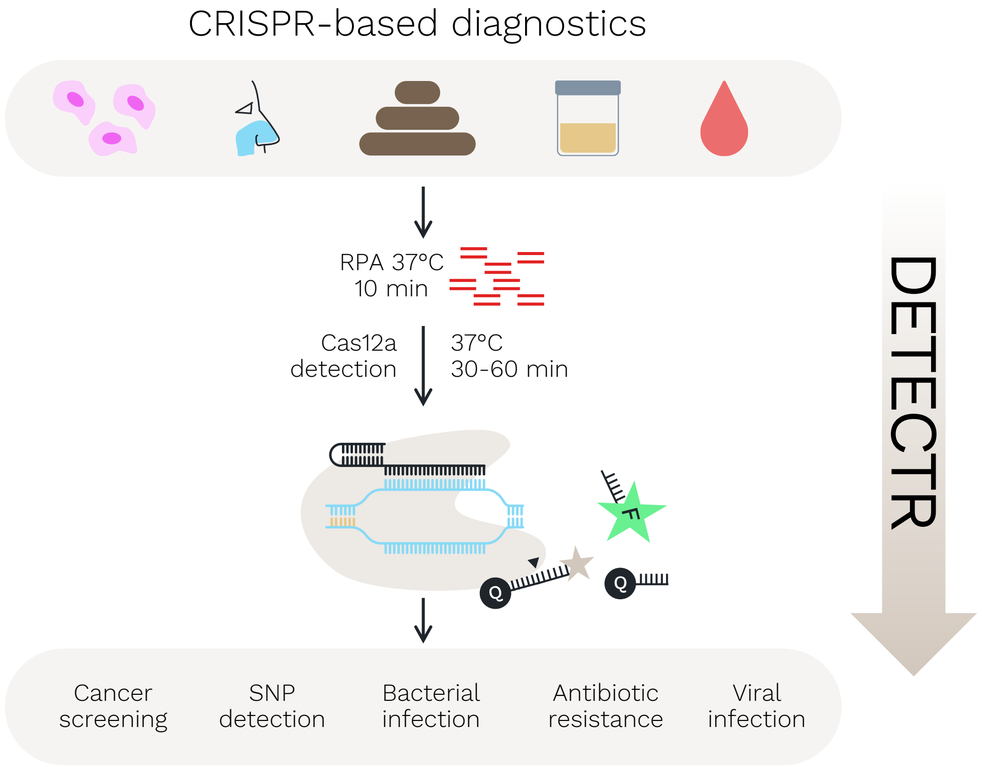

Image adapted from Chen JS, et al. ***Science.*** **2018**;360(6387):436-439.

Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; DETECTR®, CRISPR-based detection platform by Mammoth Biosciences; min, minutes; RPA, recombinase polymerase amplification; SNP, single nucleotide polymorphism; T-ALL, T-cell acute lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma.

1. Chen JS et al. Science 2018;360(6387):436-439.

Wang T. Ultrasensitive biosensor for noninvasive diagnosis of T-lymphoblastic leukemia/lymphoma. Abstract S336 presented at EHA2024.

Ultrasensitive biosensor for non-invasive diagnosis of T-lymphoblastic leukemia/lymphoma

Methods

The group tackled several strategic points to optimize the CRISPR-based biosensor system

- (1) Increase target gene recognition with an improved Cas9
- (2) Amplify the target gene with an isothermal amplification system
- (3) Establish a multiple fluorescence signal output system via the trans-cleavage ability of differently fluorescence-labeled reporter DNA/RNA by different Cas endonucleases

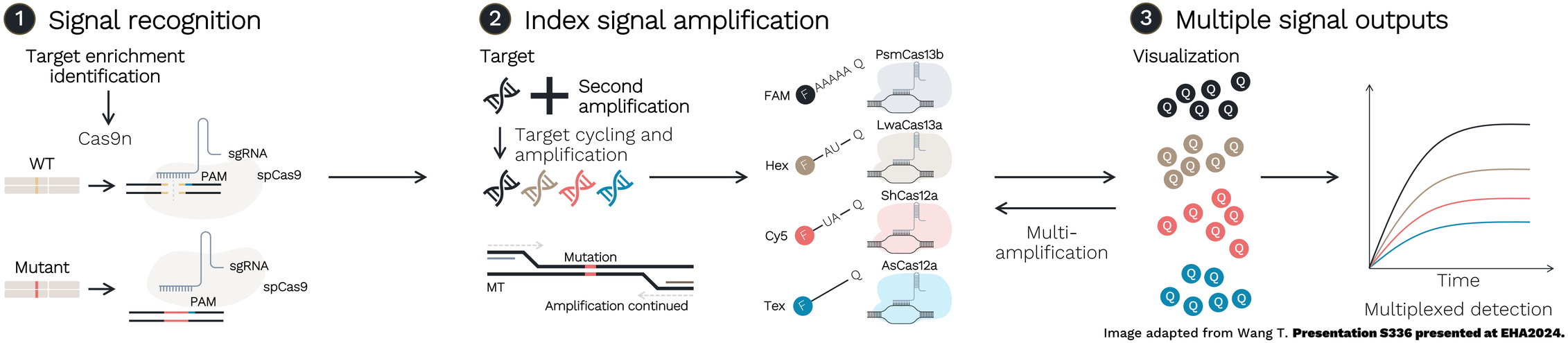

Image adapted from Wang T. **Presentation S336 presented at EHA2024.**

Optimizing all these steps, the whole process could be conducted quickly in one reaction tube throughout, delivering outcomes for several genetic biomarkers simultaneously

Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; PAM, protospacer adjacent motif; sgRNA, single guide RNA; WT, wild type.

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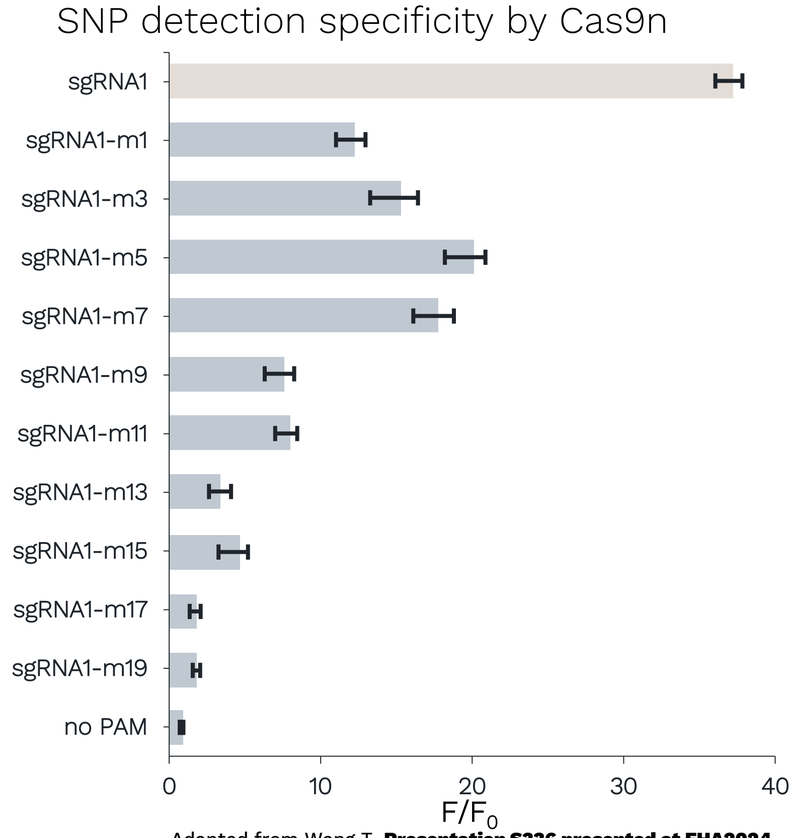
Ultrasensitive biosensor for non-invasive diagnosis of T-lymphoblastic leukemia/lymphoma

Results

- Cas9 was successfully altered via site-specific mutagenesis from standard Cas9 (with the two endonuclease domains Rucv and HNH) to Cas9n, with Rucv being inactivated by insertion of a D10A mutation
  - The Cas9n was proven to be enzymatically active and does not produce double-strand breaks but only nicks a single-strand
- Cas9 has slow off-rate and off-target activity, which affects its specificity
  - Via structure-directed mutagenesis and rational design, a novel Cas9 variant (“Hi-Fi Cas9n”) with faster kinetics and higher target specificity was engineered and proven to enhance amplification efficiency (81%)
- Cas9n is highly specific and can detect point mutations in the vicinity of PAM and the cleavage site
- Cas13a for trans cleavage was proven to be highly specific on single-base mutation discrimination as well, especially when “synthetic mismatches” were introduced in critical positions



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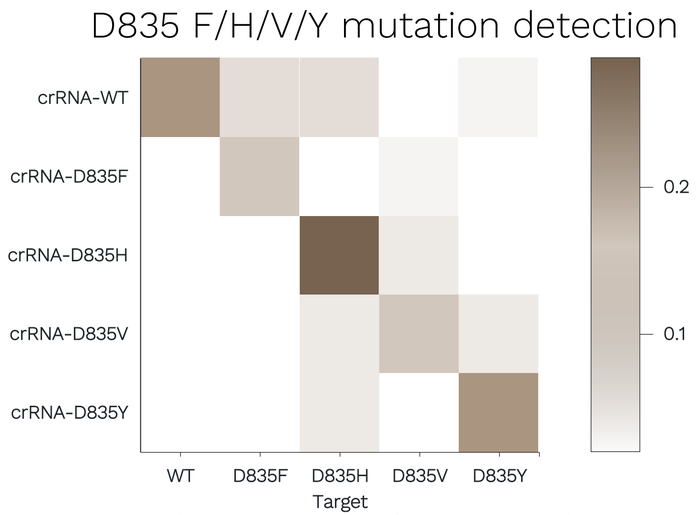
Ultrasensitive biosensor for non-invasive diagnosis of T-lymphoblastic leukemia/lymphoma

Results

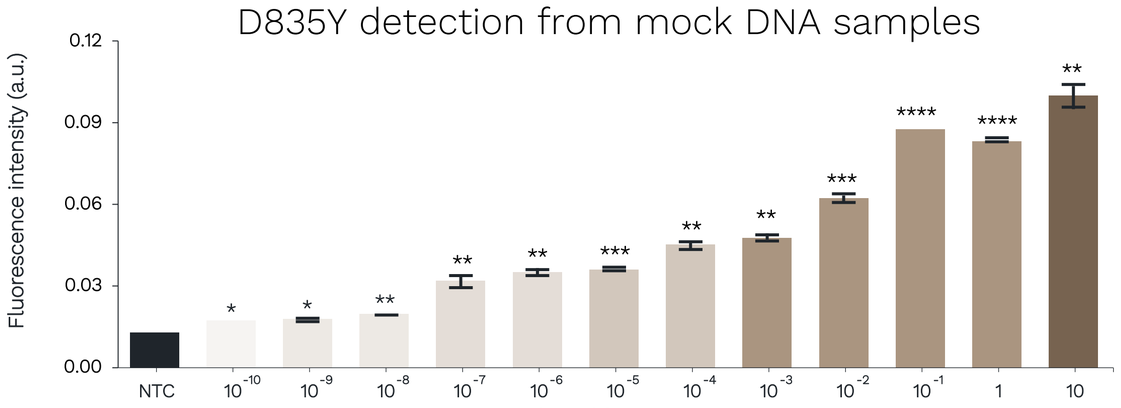
The approach allowed for the detection of specific SNVs in mock DNA samples



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

Multi-detection feasibility at high sensitivity levels

- Cas12a and Cas13a were incubated with different concentrations of corresponding fluorescence-labeled target DNA and RNA, respectively
- 0.1 aM target concentration was consistently detected = analytical LOD of 1.2 copies/reaction



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

- Multiplexed detection of different mutation samples with drug-resistant gene mutations (*IDH2*, *FLT3*, and *KRAS*) was successfully performed
- Multiple detection of further T-ALL/T-LBL-associated mutations (*NOTCH1*, *DNMT3A*, *JAK3*) are ongoing

aM, attomolar; a.u. arbitrary units; Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; crRNA, CRISPR RNA; LOD, limit of detection; NTC, no template control; SNP, single nucleotide polymorphism; T-ALL, T-cell acute lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma; WT, wild type.

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Conclusion

- CRISPR-based diagnosis can serve as approach for SNV detection in T-ALL/T-LBL
- Recent applications are time consuming, expensive, and lack multiplex abilities
- With optimized components (modified Cas9, Cas12/13) a multiplex one-step / one-pot approach seems feasible
- The adjusted method is quick, highly specific (SNP discrimination) and sensitive (LOD = 1.2 copies/reaction) for laboratory samples but needs to be validated in patient samples
- The method has the potential to be used in real-time tracking and PoC testing of high-risk gene clonal evolution and monitoring treatment response

Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; LOD, limit of detection; PoC, point of care; SNP, single nucleotide polymorphism; T-ALL, T-cell acute lymphoblastic leukemia; T-LBL, T-cell lymphoblastic lymphoma.

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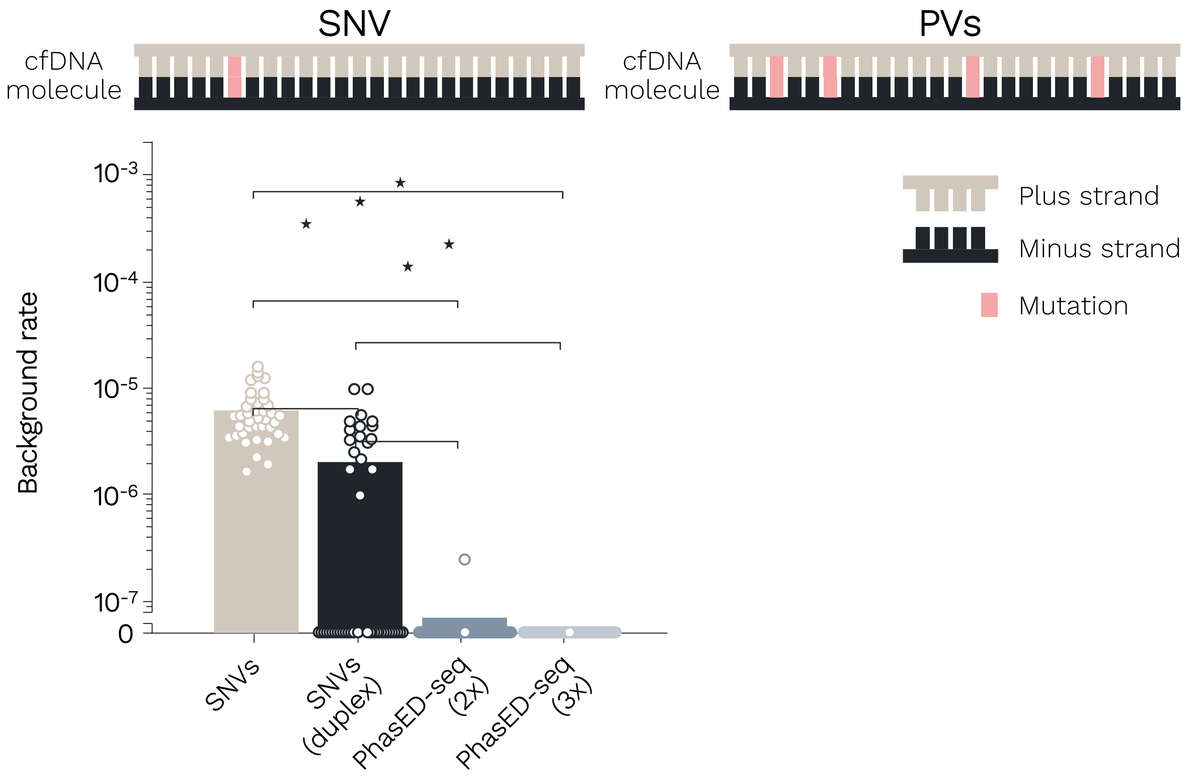
Circulating-tumor DNA (ctDNA) in diffuse large B-cell lymphoma (DLBCL): Are we ready for implementation?

PhasED-seq increases detection sensitivity in MRD testing

- Conventional MRD testing involves the detection of ctDNA via single nucleotide variants, e.g., via CAPP-seq
- PhasED-seq detects so-called 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
- Detection of phased variants significantly reduces background error rate, thereby increasing sensitivity
- PhasED-seq lowers the MRD detection limit by a factor of up to 100, compared to CAPP-seq, which could identify more patients with DLBCL who need further treatment

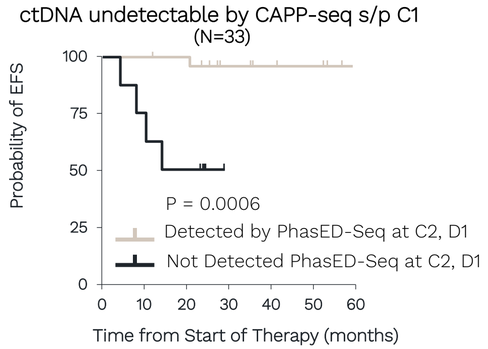


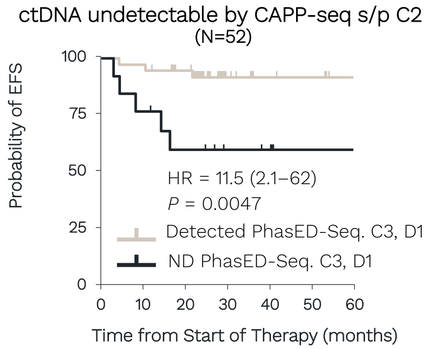
Figures adapted from Kurtz DM et al. *Nat Biotechnol.* 2021;39(12):1537-1547.
AID, Activation-induced cytidine deaminase; CAPP-seq, Cancer Personalized Profiling by deep Sequencing; ctDNA, circulating tumor DNA; DLBCL, diffuse large B-cell lymphoma; MRD, minimal residual disease; PV, phased variant; SNV, single nucleotide variant.
1. Kurtz DM et al. *Nat Biotechnol.* 2021;39(12):1537-1547.
Melani C. Circulating-Tumor DNA (ctDNA) in Diffuse Large B-Cell Lymphoma (DLBCL): Are We Ready for Implementation? Oral presentation at EHA2024

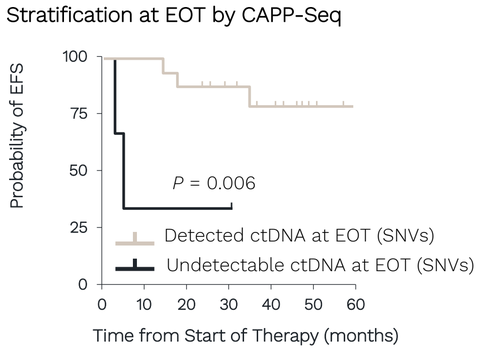
Circulating-tumor DNA (ctDNA) in diffuse large B-cell lymphoma (DLBCL): Are we ready for implementation?

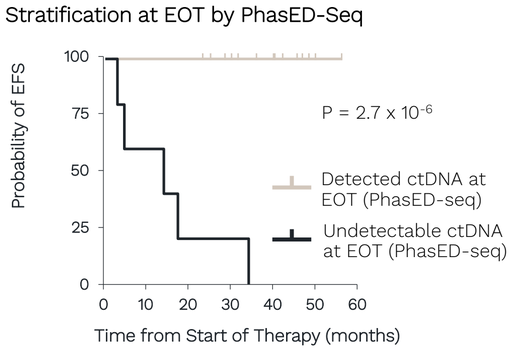
PhaseED-seq refines outcome prediction in DLBCL patients

- PhasED-seq was tested in DLBCL patients undergoing a treatment regimen of 6 cycles of chemotherapy
- Of 52 patients who tested MRD negative 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 EOT
- PhasED-seq, but not CAPP-seq, predicted event-free survival or recurrence with 100% accuracy at EOT





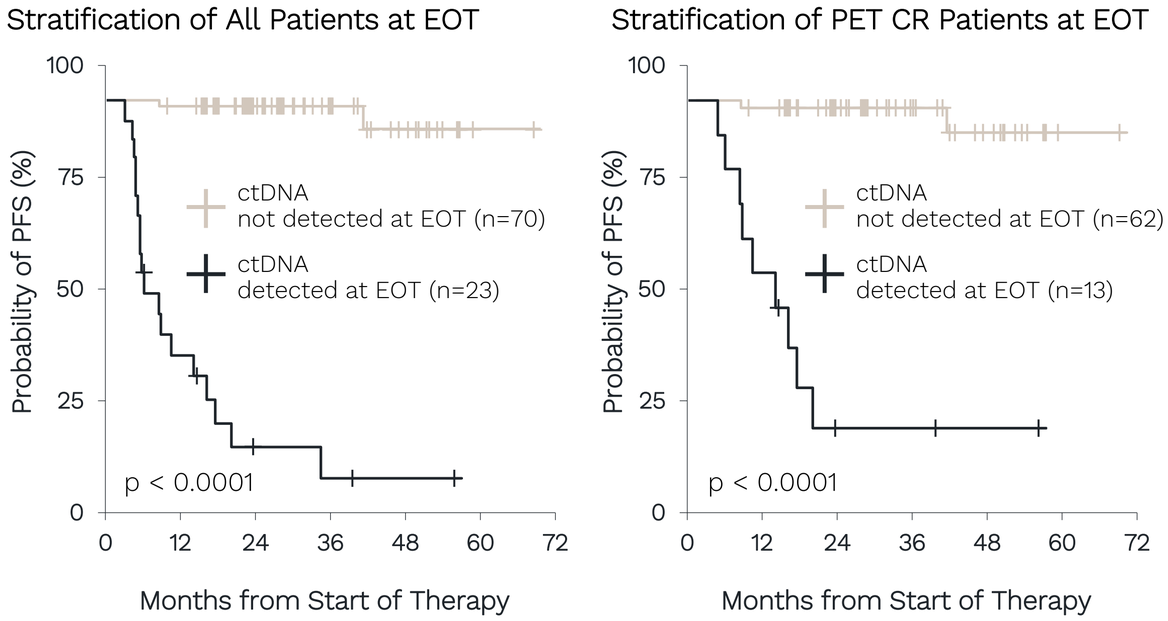




Figures adapted from Kurtz DM et al, Nat Biotechnol, 2021;39(12):1537-1547.
C, cycle; CAPP-seq, Cancer Personalized Profiling by deep Sequencing; ctDNA, circulating tumor DNA; D, day; EFS, event-free survival; EOT, end of treatment; MRD, minimal residual disease; SNV, single nucleotide variant.
1 Kurtz DM et al, Nat Biotechnol, 2021;39(12):1537-1547.
Melani C. Circulating-Tumor DNA (ctDNA) in Diffuse Large B-Cell Lymphoma (DLBCL): Are We Ready for Implementation? Oral presentation at EHA2024.

Circulating-tumor DNA (ctDNA) in diffuse large B-cell lymphoma (DLBCL): Are we ready for implementation?

MRD- per PhasED-Seq is the most prognostic factor at EOT



- In a pooled analysis of 6 clinical trials, MRD- status per PhasED-seq was more prognostic than the complete response by PET/CT
- Patients who were already stratified by CR per PET-CT could be further stratified via PhasED-seq
- 97% of patients who were tested MRD negative at EOT remained disease-free long-term (median follow-up was 17 months, 60 months for some patients)[1]

Figures adapted from Roschewski M et al. *Hematological Oncology.* 2023;41(S2):177-179.
CR, complete response; ctDNA, circulating tumor DNA; EOT, end of treatment; MRD, minimal residual disease; PET/CT, positron emission tomography/computed tomography; PFS, progression-free survival.

1 Roschewski M et al. *Hematological Oncology.* 2023;41(S2):177-179.

Melani C. Circulating-Tumor DNA (ctDNA) in Diffuse Large B-Cell Lymphoma (DLBCL): Are We Ready for Implementation? Oral presentation at EHA2024

Conclusion

- PhasED-seq uses phased variants (multiple variants occuring on one free DNA molecule) to detect MRD
- Outcome prediction is more accurate with PhaseED-seq compared to conventional, SNV-based MRD testing (e.g., CAPP-seq)
- MRD- status by PhasED-seq is more effective at stratifying patients than complete response by PET/CT

CAPP-seq, Cancer Personalized Profiling by deep Sequencing; MRD, minimal residual disease; PET/CT, positron emission tomography/computed tomography; SNV, single nucleotide variant.

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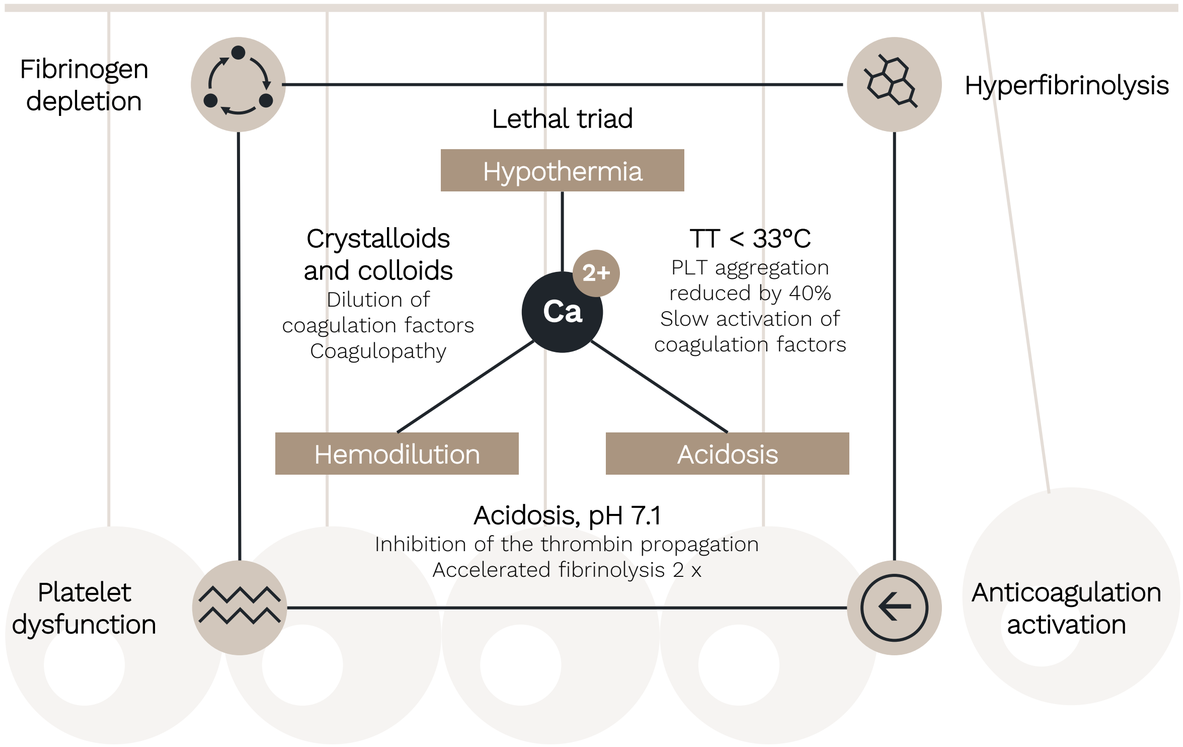
The need for rapid viscoelastic testing during coagulopathy

- Major bleeding is a frequent but often preventable cause of death after trauma or surgery
- >80% of preventable deaths are due to hemorrhage and 25% thereof are due to coagulopathy[1,2]
- Coagulopathy can occur during major injury with shock-induced endotheliopathy (SHINE): anticoagulation factors diffuse away from the wound and consume fibrinogen, thereby affecting hemostasis

The lethal triad in traumatic coagulopathy



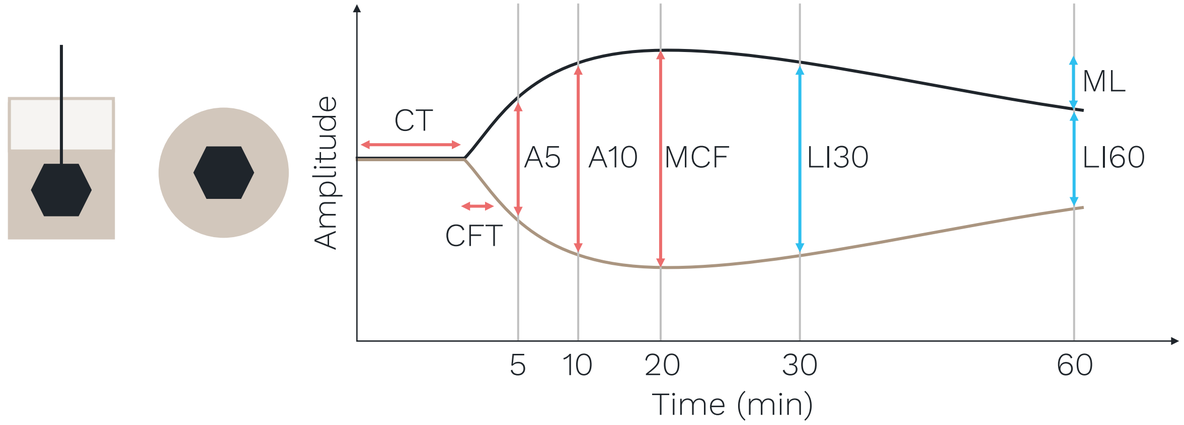
ROTEM, rotation thromboelastometry; TEG, thromboelastography.

1. Callcut RA, et al. *J Trauma Acute Care Surg.* 2019;86(5):864-870. 2. Eastridge BJ, et al. *Transfusion.* 2019:59(S2):1423-1428.

Nešković N. Use of point of care testing (ROTEM) in coagulopathy. Oral presentation at EHA2024.

TEG/ROTEM to test viscoelasticity

- Standard laboratory coagulation testing is too slow in emergencies.
  - However, viscoelastic testing of a blood sample yields first results after 10-15 minutes
- TEG/ROTEM 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
  - Important time points: 5 and 10 minutes (A5, A10), time point of maximum clot firmness, clot lysis (LI30, LI60), maximum lysis[1]
- 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



Various assays of viscoelastic tests enable insight into extrinsic and intrinsic coagulation pathways

EXTEM	FVII, FX, FV, FII, fibrinogen, platelets, fibrinolysis
INTEM	FXII, FXI, FIX, FVIII, FX, FV, FII, FI, fibrin, platelets, fibrinolysis
FIBTEM	Contribution of fibrinogen to the clot formation
APTEM	Inhibition of fibrinolysis, comparison to EXTEM
HEPTEM	Heparin inactivation, comparison to INTEM

A10, 10-minute timepoint in ROTEM; MCF, maximum clot firmness; ROTEM, rotation thromboelastometry; TEG, thromboelastography.

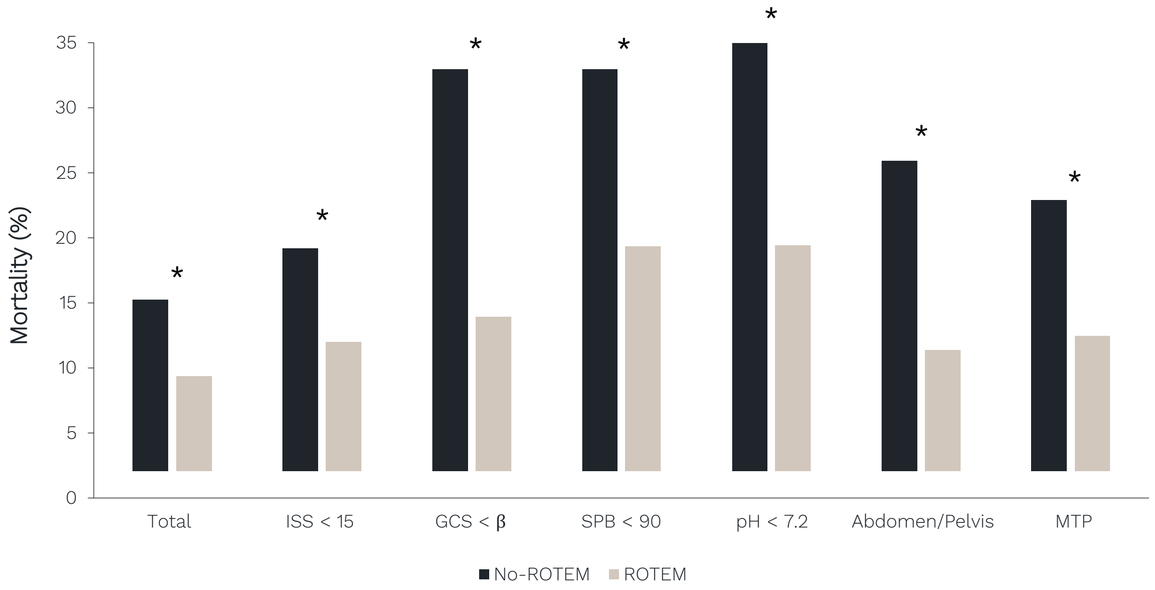
1 Lang et al., *Hemostaseologie* 2006; 26(3 Suppl 1):S20-9.
Nešković N. Use of point of care testing (ROTEM) in coagulopathy. Oral presentation at EHA2024

Using ROTEM-guided resuscitation in coagulopathy

- 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[1]
- Fibrinogen level is an independent predictor of patient outcome with major bleeding[2]
- ROTEM/ TEG can guide the timing of platelet, plasma and red blood cell transfusion, and the needed ratio between these components[3,4]
- ROTEM-guided resuscitation led to decreased mortality compared to no ROTEM-guidance (7.3 vs. 13.1% overall) in trauma patients[5]

	ROC AUC (95% CI)	Cut off value	Sensitivity	Specificity
FIBTEM MCF	0.84 (0.79-0.88)	≤ 7 mm	77.5 %	74.9 %
FIBTEM A10	0.83 (0.78-0.87)	≤ 4 mm	63.3 %	83.2 %
Fibrinogen concentration	0.83 (0.78-0.87)	≤ 1.48 g/L	84.2%	68.3 %

Mortality rates stratified by groups in trauma patients with and without POC guide resuscitation



A10, 10-minute timepoint in ROTEM; MCF, maximum clot firmness; ROTEM, rotation thromboelastometry.

1. Schoechl H et al., *Crit Care.* 2011; 15(6):R265. 2 Inaba K et al., *J Am Coll Surg*. 2013; 216(2):290-7. 3 Inaba K et al., *J Trauma Acute Care Surg*. 2015 78(6):1220-9. 4 Rossaint R et al., *Crit Care* 2023; 1;27(1):80. 5 Lammers et al, *J Trauma Acute Care Surg* 2020;89(1):145-152

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Conclusion

- Major bleeding is the most common preventable cause of death in trauma patients and caused by coagulopathy in 25% of cases
- ROTEM/ TEG as a point-of-care test offers a quick assessment of coagulation status in patients with major trauma or risk of major bleeding
- ROTEM and its variants can guide the timing and ratio of transfusion products with a major impact on mortality

ROTEM, rotation thromboelastometry; TEG, thromboelastography.
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07

Appendix

Abbreviations

A1AT	alpha-1 antitrypsin
AE	adverse event
AESI	adverse events of special interest
Ag	antigen
AI	artificial intelligence
AID	Activation-induced cytidine deaminase
aM	attomolar
AML	acute myeloid leukemia
ANC	absolute neutrophile count
AsCas12a	Acidaminococcus sp. clustered regularly interspaced short palindromic repeats-associated protein 12a
AT	antithrombin
ATAC	Assay for Transposase-Accessible Chromatin
AUC	area under the ROC curve
B-ALL	B-cell acute lymphocytic leukemia
BCL11A	B-cell lymphoma/leukemia 11A
BCL2	B-cell lymphoma 2
BCL2i	B-cell lymphoma 2 inhibitor
BCMA	B-cell maturation antigen
BLI	bioluminescent imaging
BM	bone marrow
bp	base pair
BTK	Bruton's tyrosine kinase
CAPP-seq	Cancer Personalized Profiling by deep Sequencing
CAR T	chimeric antigen receptor T-cell
CAR	chimeric antigen receptor
CARPOOL	pooled CAR-T-cell therapy
Cas	CRISPR-associated protein
Cas9	CRISPR-associated protein 9
cBTKi	covalent Bruton's tyrosine kinase inhibitor
cCART	classical chimeric antigen receptor T-cell
CD	cluster of differentiation
ChIP	chromatin immunoprecipitation
Chr	chromosome
CI	confidence interval
CIT	chemo-immunotherapy
CITE-seq	Cellular Indexing of Transcriptomes and Epitopes by sequencing
CLL	chronic lymphocytic leukemia
c-Myc	cellular myelocytomatosis oncogene
CNS	central nervous system
CNV	copy number variation
CR(i)	complete response (with incomplete count recovery)
CR	complete response
CRc	composite complete remission (CR/CRi)
CRISPR	clustered regularly interspaced short palindromic repeats
CRR	complete response rate
crRNA	CRISPR RNA
CRS	cytokine release syndrome
CTC	circulating tumor cell
ctDNA	circulating tumor DNA
dCas9	dead Cas9
DC-SIGN	dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DETECTR®	CRISPR-based detection platform by Mammoth Biosciences; min, minutes;
RPA	recombinase polymerase amplification
DL	dose level
DLBCL	diffuse large B cell lymphoma
DLT	dose limiting toxicity
$Dmax_{bulk}$	maximum distance between the target lesion and another lesion
DOR	duration of response
DSB	double-strand break
ECM	extracellular matrix
ECOG PS	Eastern Cooperative Oncology Group performance status
EFS	event-free survival
EMA	European Medicines Agency
EMD	extramedullary disease
EOT	end of treatment
EV	extracellular vesicle
exa-cel	exagamglogene autotemcel
FACS	Fluorescence-Activated Cell Sorting
FAP	fibroblast activation protein
FBC	full blood count
FBG	fibrinogen
FC	fludarabine and cyclophosphamide
FCS	flow cytometry standard
FDA	U.S. Food and Drug Administration
FDC	follicular dendritic cell
FISH	fluorescence in situ hybridization
FL	follicular lymphoma
FLT3	Fms-like tyrosine kinase 3
FRC	fibroblastic reticular cell
GAN	generative adversarial networks
GPRC5D	G-protein coupled receptor family C group 5 member D
GvHD	graft-versus-host disease
H3K27Ac	histone H3 lysine 27 acetylation
Hb	hemoglobin
HbA	hemoglobin A
HbE	hemoglobin E
HbF	fetal hemoglobin
HBG	hemoglobin
HbS	sickle hemoglobin
HCT	hematocrit
HD	high dimensional
HDR	homology-directed repair
HLH	hemophagocytic lymph histiocytosis
HNF-4	hepatocyte nuclear factor 4
HO	hepatic organoid
HPFH	hereditary persistence of fetal hemoglobin
HR	hazard ratio
HSCT	hematopoietic stem cell transplantation
IBEX	Iterative Bleaching Extends multi-pleXity
ICANS	immune effector cell-associated neurotoxicity syndrome
IF	immunofixation
IFNγ	interferon gamma
IMWG	International Myeloma Working Group
InDel	insertion/deletion
iPET	interim PET
iPSC	induced pluripotent stem cell
IRd	lenalidomide + dexamethasone + ixazomib
ISS	International Staging System
ITD	internal tandem duplication
iwCLL	international workshop on CLL
KLF	Krüppel-like factor
LAG3	Lymphocyte-activation gene 3
LBL	lymphoblastic lymphoma
LC	liquid chromatography
LD	lymphodepletion
LDH	lactate dehydrogenase
LIFEx	Local Image Features Extraction
LINCS	Library of Integrated Network-Based Cellular Signatures
LNP	lipid nanoparticle
LOD	limit of detection
LoT	lines of treatment
LRF	leukemogenic transcriptional repressor factor
m/z	mass to charge ratio
M+IC	midostaurin plus immunochemotherapy

Abbreviations

mAbs	monoclonal antibodies
MAD	maximum administered dose
MCF	maximum clot firmness
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCL	mantle cell lymphoma
MCV	mean corpuscular volume
mDOR	median duration of response
MDS	myelodysplastic syndrome
MGUS	monoclonal gammopathy of undetermined significance
MHC	major histocompatibility complex
ML	machine learning
MLH1	MutL protein homolog 1
MM	multiple myeloma
MRD	minimal residual disease
MRDneg	MRD negativity
mRNA	messenger RNA
MS	mass spectrometry
MTD	maximum tolerated dose
MTV	metabolic tumor volume
MZL	marginal zone lymphoma
nCas9	nickase Cas9
NGF	next-generation flow
NGS	next-generation sequencing
NHEJ	non-homologous end joining
NHL	non-Hodgkin's lymphoma
NK	natural killer
NPM1	nucleophosmin
nPR	nodular partial response
NTC	no template control
Oct4	octamer-binding transcription factor4
ODAC	Oncologic Drugs Advisory Committee
ONE-seq	OligoNecleotide Enrichment and sequencing
ORR	overall response rate
OS	overall survival
PAM	protospacer adjacent motif
PB	peripheral blood
PBMC	peripheral blood mononuclear cell
PCA	principal component analysis
PCNSL	primary CNS lymphoma
PD	progressive disease
PET	positron emission tomography
PET/CT	positron emission tomography / computed tomography
PFS	progression-free survival
PLT	platelet count
PoC	point of care
PR	partial response
PRD	peripheral residual disease
PR-L	partial response with lymphocytosis
PSC	pluripotent stem cell
PV	phased variant
QD	once daily
R/R	relapsed/refractory
RBC	red blood cell
RBC-TI	rate of red blood cell transfusion independence
Rd	lenalidomide + dexamethasone
Reni-cel	renizgamglogene autogedtemcel
rh	recombinant human
RNA	ribonucleic acid
RNP	ribonucleoprotein
ROTEM	rotation thromboelastometry
RP2D	recommended phase 2 dose
SAE	serious adverse event
sc	single-cell
SCD	sickle cell disease
scFv	single chain variable fragment
scMRD	single-cell MRD
SCNSL	secondary CNS lymphoma
sCR	stringent complete response
SD	stable disease
sgRNA	single guide RNA
shRNA	small hairpin RNA
SLL	small lymphocytic lymphoma
SMM	smoldering multiple myeloma
SNP	single nucleotide polymorphism
SNV	single nucleotide variant
SoC	standard of care
SOX2	SRY-box transcription factor 2
ssCART	small hairpin RNA element to silence the interleukin-6 (IL-6) gene chimeric antigen receptor T-cell
SUVpeak	peak standardized uptake value
T-ALL	T-cell acute lymphoblastic leukemia
tBE	transformer base editor
TCR	T-cell receptor
TDT	transfusion-dependent thalassemia
TEAE	treatment-emergent adverse event
TEG	thromboelastography
TEV	tobacco etch virus
TF	transcription factor
Tim	T-cell immunoglobulin and mucin-domain containing-3
T-LBL	T-cell lymphoblastic lymphoma
TLS	tumor lysis syndrome
TME	tumor microenvironment
TNFα	tumor necrosis factor alpha
TRAC	T-cell receptor alpha constant
TRAE	treatment-related adverse event
t-SNE	t-distributed stochastic neighbor embedding
UMAP	uniform manifold approximation and projection
VGPR	very good partial response
VOE	vaso-occlusive event
WBC	white blood cell
WGS	whole genome sequencing
WGTS	whole genome and transcriptome sequencing
WM	Waldenstrom macroglobulinemia
WT	wild type
WTS	whole transcriptome sequencing

OS, overall survival

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