ALL relapse after anti-CD19 CAR-T cells therapy in a young adult: which therapeutic options?

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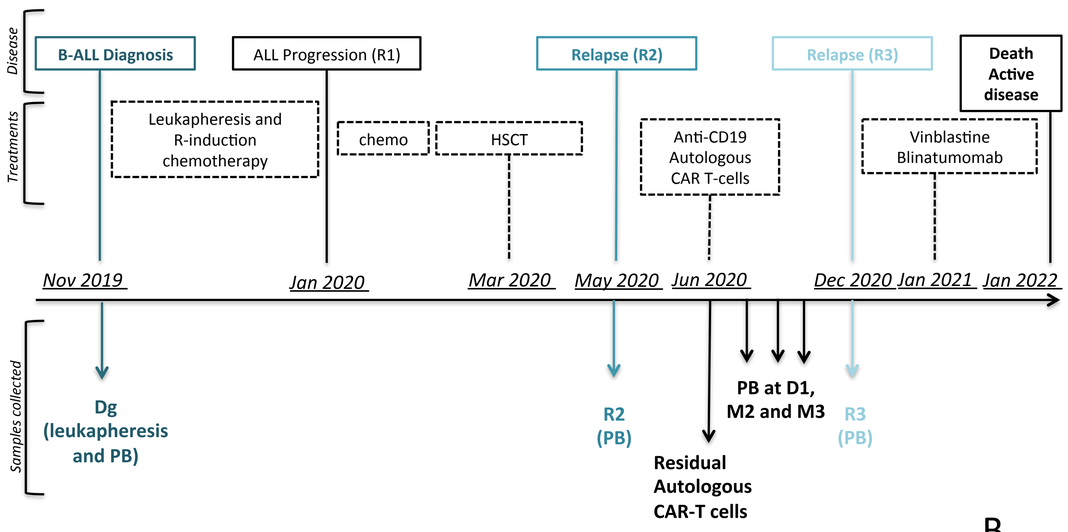
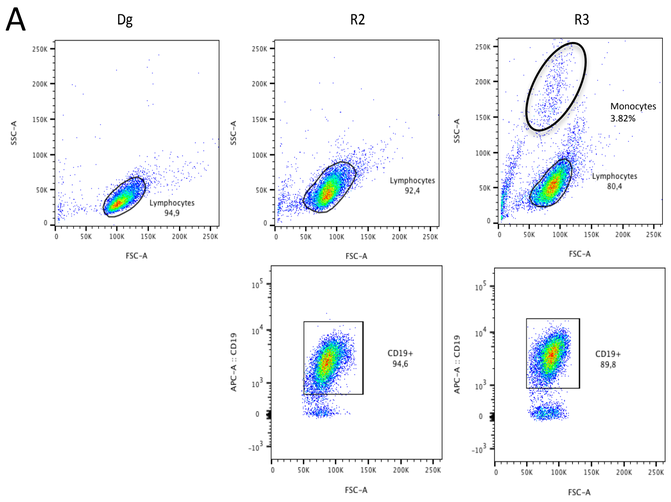
Introduction:

T-cells expressing a Chimeric Antigen Receptor (CAR) recognizing the CD19 antigen allowed high early response rates in high-risk B acute lymphoblastic leukemias (B-ALL). Nevertheless, 35% to 44% of patients relapse. Among the mechanisms of immune evasion in CD19$^+$ relapses of ALL, expression and secretion of molecules inhibiting cytotoxic T-lymphocytes, or lack of expression of co-stimulatory molecules, have been described. Expansion, persistence and cytotoxic activity of CAR-T cells could also be deficient.

Here, we present the case of a young adult who presented successive relapses of ALL after chemotherapy, hematopoietic stem cell transplant (HSCT) and anti-CD19 CAR-T cells. An extended immunophenotype of leukemic cells and sensitivity to anti-CD19 CAR-T cells mediated lysis are analysed.





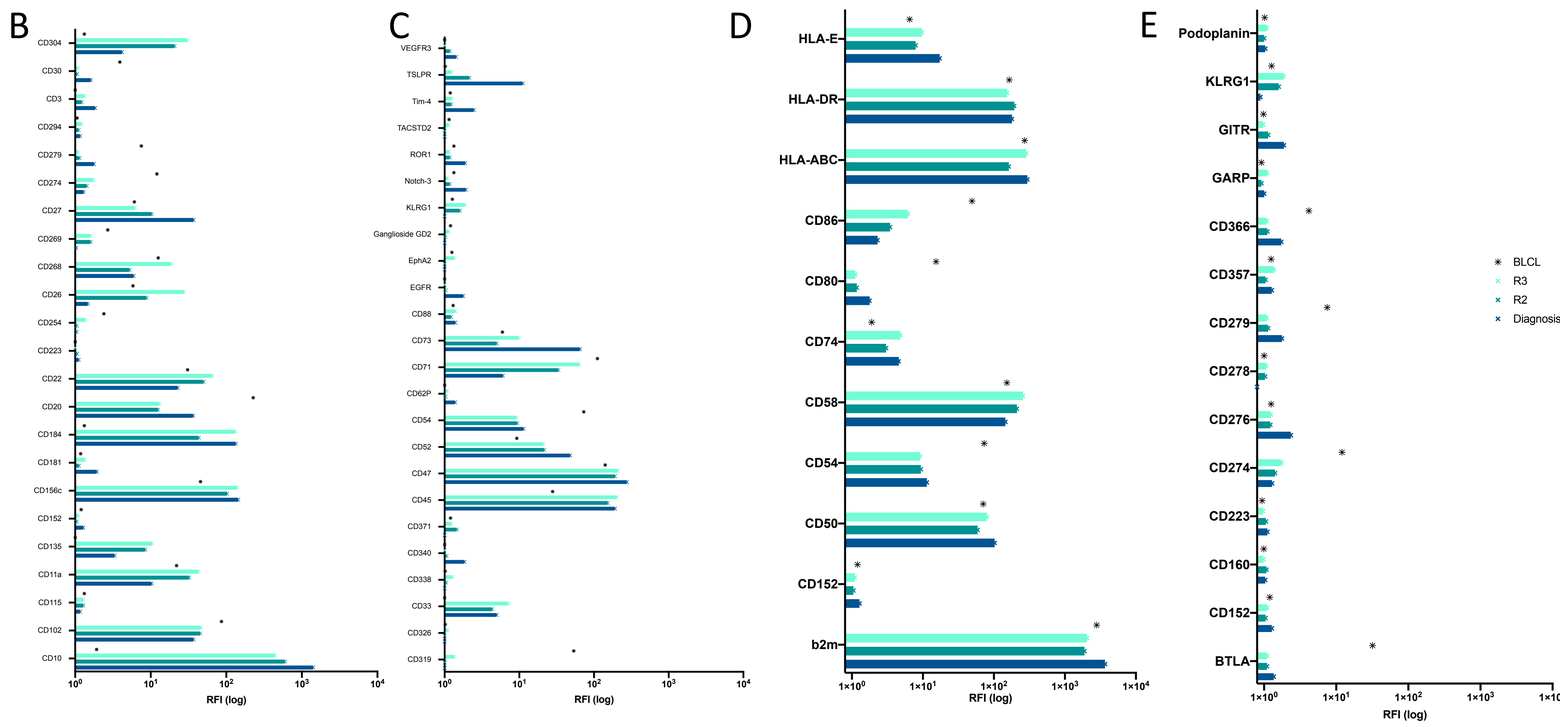
Cells: At Dg, peripheral blood cells were collected from the leukapheresis product. Peripheral blood samples were collected at R2 and R3 relapses and peripheral blood mononucleated cells (PBMC) were isolated by density gradient centrifugation on FICOLL-Paque solution and analysed by flow cytometry.

Morphologic Dot plot of peripheral blood cells isolated at Dg, R2 and R3 relapses and used for phenotype analysis (A), showed on R3 sample appearance of monocytic population which was secondly analysed and was CD14$^+$, HLA-DR ^{neg}, CD33$^+$, CD11b ^{high}. This monocytic population recently emerged as tumor-induced immunosuppression mediator.

Clinical case:

A 21-years-old patient presented with a hyperleukocytic, CRLF2 overexpressing and IKZF1 deleted, B-ALL (Dg) in November 2019. A relapse (R2) occurred 2 months after HSCT and the patient received an anti-CD19 CAR-T cells (tisagenlecleucel) in July 2020.

Detection of circulating CAR-T cells in blood showed an expansion peak at day 8 (3.5% of T lymphocytes) and the appearance of CD14$^+$/HLADR$^{lo/neg}$ monocytic cells **(A)**. A second CR was obtained with negative minimal residual disease. The patient presented a loss of B cells aplasia at 4.5 months and a CD19$^+$ relapse at 5.5 months post-CAR-T cells (R3). He received palliative chemotherapy and deceased in January 2022 .

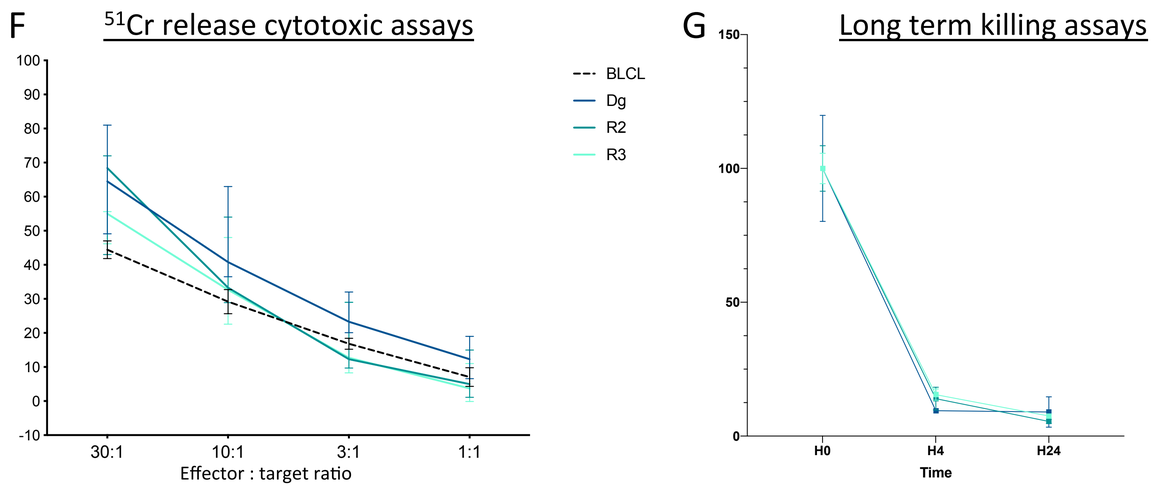


Phenotype: An extended immunophenotype of Dg, R2 and R3 cells, was performed using the Human Cell Surface Marker Screening Kit from Biolegend® (LEGENDscreen). Cells were first incubated with an anti-CD19-APC coupled antibody (Biolegend®, clone HIB19) in order to gate the leukemic blasts for analysis. Cells were acquired on CANTO II cytometer (BD Biosciences) (Plateforme CytoCell – Nantes University).

CD19 expression decreased between Dg and R2 and R3 respectively, as CD20, while CD22 was highly expressed in all samples.

Interestingly, new targetable antigens appeared as CD135 (*FLT3*) (present on only 7% of the ALL cells at diagnosis, and on 100% of cells in R2 and R3). Similarly CD268 (BAFF-R), CD304 (Neuropilin-1) and CD71 expressions increased in relapse ALL cells (B,C).

Expression of molecules involved in immunological synapse (D) remained stable in all samples. Co-stimulatory ligands expression seem stable from Dg to R2 and R3 (D). Expression of checkpoint inhibitors was low in all samples (E).



Cytotoxic assays:

^{51}Cr assays

Target cells were labeled with 75µCi ^{51}Cr for one hour and then plated with effector T cells at the indicated effector-to-target ratio (E:T ratio). After a 4-hour incubation at 37°C, released ^{51}Cr activity was counted in a scintillation counter (PerkinElmer).

Long term killing assays

Sensitivity to CAR-T induced lysis was also analysed over 24 hours, because it better represents the in-vivo CAR-T activity. ALL cells and effectors were plated at the E:T ratio 3:1 and incubated at 37°C. Cells were collected at different time points: 0h, 4h and 24h of co-culture and labelled with a Fixable Viability Stain 780 (BD Biosciences), a PE-coupled anti-CD22 (Dako) and FITC-coupled anti-CD3 (Beckman Coulter) antibodies, so as to distinguish ALL (CD22$^+$) residual viable cells by flow cytometry.

We observed that all ALL, even R3, were similarly lysed by anti-CD19 CAR-T cells (F). The lysis scores were directly proportional to the effector/target ratio. The rapid and efficient lysis of all ALL samples was confirmed by the *long term killing assays* with only 0,5% residual viable CD22$^+$ cells (ALL cells) after 4 hours of co-culture (G).

In conclusion, in these successive ALL relapses, no resistance to anti-CD19 CAR-T mediated lysis could be observed, while new targetable antigens were revealed. Additional targeted therapy or second anti-CD19 CAR-T infusion could then have been discussed. Nevertheless, modulation of immunosuppressive microenvironment, as targeting CD14$^+$/HLA-DR$^{lo/neg}$ monocytes, might offer a way to improve CAR-T cell therapy.