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Altered growth in KMT2A/ENL induced human B cells from peripheral blood

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Abstract

Introduction Leukemia, like most cancers, likely originates from a unique genetic defect. Accessible models that accurately reflect this uniqueness for studying cellular characteristics and responses are still lacking. The *KMT2A/MLLT1* fusion gene (encoding the KMT2A/ENL fusion protein) is a common chromosomal translocation in acute leukemia and demonstrates strong oncogenic potential. *KMT2A* is also referred to as mixed lineage leukemia (*MLL*).

Objective The aim of this study was to generate leukemia-like B-cells from human adult cells by transfecting KMT2A/MLLT1 fusion gene into healthy PBMCs.

Method The *KMT2A/MLLT1* fusion gene was inserted into peripheral blood mononuclear cells (PBMCs) isolated from three healthy individuals. Triplicate experiments were performed using the lipofection method. Flow cytometry confirmed induced cells by detecting expression of the KMT2A/ENL (MLL +) fusion protein and characterized B-cell leukemia markers (CD19, CD34, CD45 and CD38).

Results MLL + CD45 + cells significantly increased to 45.7 ± 19.5% (N = 9) in transfected cells on day 10. The mean fluorescence intensity of CD45, CD34 and CD38, however, remained unchanged. Interestingly, unlike untransfected cells, the percentage of MLL + CD19 + (CD45 + CD38 +) B cells also significantly increased by day 10 (35.4 ± 32.0% vs 3.7 ± 2.4%) demonstrating abnormal growth characteristics.

Conclusions These rapid, accessible and reproducible KMT2A/ENL + leukemia-like cells may serve as a suitable model for studying leukemia.

Keywords KMT2A/ENL, Leukemia model, Chromosomal translocation

Background

Cancer is recognized as a genetic disease caused by variants, mutations and other alterations that may have accumulate over time (National Institutes of Health). In the 2022 World Health Organization (WHO) classification of hematopoietic and lymphoid tissues, multiple approaches were used to describe and define diseases including clinical features, morphology, immunophenotype, and genetic data [1]. Advances in molecular genetics has facilitated the discovery of genetic lesions in these malignancies, with some findings representing dramatic shifts. For example, an early report stated that "no cytogenetic abnormality is specific for hairy cell leukemia", whereas later studies identified the unique BRAF V600E mutation

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as a diagnostic hallmark present in nearly all patients with this condition. These genetic signatures are valuable diagnosis of disease, risk stratification and treatment selection. They also serve as predictive factors and provide the molecular basis novel therapeutic approaches based on the principles of precision medicine [2].

Leukemia is a cancer of the blood and bone marrow leads to uncontrolled production of abnormal white blood cells, typically arising from a single lineage such as B cells. Lymphoma is a related malignancy in which abnormal cells originate in the lymphatic system, including the lymph nodes and associated tissues. Leukemias are broadly categorized as acute or chronic leukemia, with both lymphoid/lymphoblastic and myeloid/myeloblastic lineages affected. The majority of acute, chronic and other less common forms of leukemia originate from B cells [3] and are collectively referred to here as B-cell leukemias.

Genetic aberrations involving *KMT2A* (11q23), formerly known as *MLL, HRX,* or *ALL1,* were among the first four recurrent genetic abnormalities identified in the classification of acute myeloid leukemia (AML) [4]. Chromosomal rearrangements of the human *KMT2A/MLL* gene are associated with both de novo and therapy-related acute leukemias in infants, children, and adults. These can also manifest as mixed-lineage leukemia, which expresses both lymphoid and myeloid markers. *KMT2A* translocations can occur with as many as 107 different partner genes [5]. The AML subtypes that result from these various rearrangements and aberrations of MLL are not identical; and cases involving translocations, disease biology is strongly influenced by the partner gene.

KMT2A rearrangements (KMT2A-r) are important diagnostic and prognostic markers included in classification of acute lymphoblastic leukemia/lymphoma [6]. KMT2A-r occur in approximately 9% of adult cases, 3–5% of pediatric cases and 61–80% of infant cases of acute lymphoblastic leukemia (ALL). They also account for 5–11% of adult cases, ~ 15% of pediatric cases, and 33–75% of infant cases of acute myeloid leukemia (AML) (reviewed in [7]).

MLL rearrangements in ALL are associated with a poor [8] to moderate prognosis, often accompanied by a high risk of relapse [9]. Importantly, the partner gene strongly influences patient outcomes in cases of MLL rearrangement [8]. Because cytotoxic chemotherapy regimens remain unsatisfactory, numerous clinical trials are investigating targeted KMT2A-r therapies, including menin inhibitors, which have progressed to Phase I/II trials and show potential for improving patient responses [10].

The three most frequent translocation partner genes (TPGs) of the *KMT2A* gene are *AFF1* or *AF4, MLLT3* or *AF9* and *MLLT1* accounting for approximately 37.1%, 18.0% and 13.5% of TPGs, respectively [5]. The distribution of these KMT2A-r events among acute leukemias is heterogeneous. Most *AF4* (99.3%) and *MLLT1* (89.7%) translocations occur in ALL, whereas *AF9* translocations are more evenly distributed between ALL (43.8%) and AML (56.6%). Regarding age distribution, most *AF4* (43.3%) and *AF9* (28.8%) cases are seen in adult ALL, although *AF9* is somewhat more evenly distributed. In contrast, the majority of *MLLT1* (52.5%) cases are concentrated in infant ALL. Advances in next-generation sequencing (NGS) technology have now enabled detection of KMT2A-r in nearly all cases (100%) [5].

Mutations involving *KMT2A* are also observed in mature hematolymphoid tumors. For example, *KMT2A*-rearranged diffuse large B cell lymphoma (DLBCL) has been reported in both adult and pediatric patients. While adult patients responded well to conventional chemotherapy, pediatric cases required more intensive treatment to achieve remissions [11].

Due to its biology, treatment outcome, and oncogenicity, with *MLL* fusions representing early leukemogenic events that typically do not require additional genetic changes to induce leukemia, this translocation has been the subject of numerous studies aimed at creating disease models. Such models are developed to explore pathogenic mechanisms and to test alternative therapeutic strategies. The best models should recapitulate at least the key attributes of the human disease [12]. An ideal model should be relevant to human disease (particularly reflecting pathophysiology, progression of disease, and clinical symptoms), consistent, reproducible and ethically appropriate for either in vivo or in vitro applications. Example of disease models include immortalized cell lines and human induced pluripotent stem cells (in vitro), as well as invertebrate models such as *Caenorhabditis elegans* and *Drosophila,* and vertebrate models such as zebra fish, rodents and non- human primate (in vivo) [13].

The advent of molecular cloning has enabled researchers to express fusion genes from the many leukemia-associated chromosomal translocations in the bone marrow of model animals. The bone marrow retroviral transduction/transplantation model has been the most frequently used in vivo method for expressing MLL fusion proteins and has successfully replicated the original diseases in transgenic mice. The experiments have provided critical insights into protein interactions, microenvironment signaling, and epigenetic regulation, as well as identified novel therapeutic targets that are now being evaluated in clinical trials [14].

Target cells (hematopoietic stem and progenitor cells (HSPCs) have been sourced from bone marrow, embryonic stem cells, and cord blood cells, and subsequently

transplanted into immunodeficient mice. While this approach offers many advantages, it also has certain limitations and disadvantages. Natural packaging constraints restrict the size of retroviral genomes [15] and, consequently, the size of retroviral plasmids. Another drawback is viral integration into tumor suppressor or oncogenes loci, which can lead to secondary malignancies. To address these issues, knock-in chimeric mice were generated through homologous recombination, enabling transgene expression from the endogenous locus [16]. More recently, genome-editing technologies such as transcription activator-like effector nucleases (TALENs) [17] and clustered regularly interspaced short palindromic repeats (CRISPR) [18] have been employed, making it possible to alter genes in situ.

B cell malignancies are diverse. Numerous mouse models with altered expression of cancer-driving mutations in B-cell tumorigenesis have been developed in genetically modified mice to investigate complex neoplastic gene functions and evaluate new therapeutic targets [19]. However, these models do not always accurately recapitulate the corresponding human diseases.

Human leukemia cell lines are invaluable research tools with many advantages. However, the number of available B-cell leukemia lines remains limited. A major drawback of immortal cells is that they may fail to maintain the characteristics of primary cells. For example, genetic instability can arise from serial passaging, cross-contamination with other cell lines, or mycoplasma infection, all of which can alter gene expression and cell behavior [20]. Gene-environment interactions are increasingly important for understanding the etiology of leukemia. Mouse models have been widely used to study the multistage process of leukemogenesis and involvement of additional chromosomal translocations, such as ETV6-RUNX1, E2A-PBC1, BCR-ABL and others [21].

In this experiment, we aimed to induce expression of the *KMT2A/ENL* fusion gene in peripheral blood cells from healthy individuals to generate leukemia-like cells.

Methodology

Peripheral blood samples

Approval to conduct this study was obtained from the Ethics Committee for Research Involving Human Subjects (JKEUPM), Universiti Putra Malaysia, and the National Medical Research Ethics Committee Registry (MREC), Ministry of Health Malaysia (NMRR-17-2697-38131). Three subjects were recruited from the Otolaryngology Department, Hospital Serdang, and the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Adults (>18 years old) of both sexes were included in the study. Peripheral blood samples were collected by a qualified medical personnel. All procedures adhered to the principles of the Declaration of Helsinki.

A 20 mL blood sample was collected from each volunteer, and mononuclear cells were isolated by gradient centrifugation using Ficoll-Paque PLUS according to manufacturer's instructions (GE Healthcare, Sweden). For long-term storage, cells were resuspended in freezing medium (10% of DMSO, 40% of FBS and 50% of RPMI 1640; Gibco, Invitrogen, USA) and stored in liquid nitrogen after overnight slow cooling to −80 °C. To reduce contamination, samples were processed in a Class II Biosafety Cabinet (Telstar, Spain) under sterile conditions.

Generation of *KMT2A/MLLT1* expressing cells

Synthesis of KMT2A/MLLT1 fusion gene

The sequence for t(11;19)(q23; p13.3), corresponding to the *KMT2A/MLLT1* fusion gene (1683 bp) (Supplementary Figure S1A) was taken from Buechele et al. (2015) [17]. Synthesis of the sequence was outsourced to Apical Sdn. Bhd. and cloned into an IDT cloning vector with a minor modification: the addition of two restriction enzyme sites (BamHI and XbaI) at the sequence termini (Supplementary Figure S1B). The sequence was validated and confirmed by the company. The p65mNeonGreen plasmid (plasmid #127,172, Addgene, USA) was used as positive control.

Amplification of plasmid

The IDT (Intergrated DNA Tecnologies) vector containing the *KMT2A/MLLT1* fusion gene was transformed into Top10 competent cells (Invitrogen, USA) using the heat shock method. After a 30 min incubation of 1 µg plasmid DNA with the competent cells, heat shock was performed by exposing the mixture to 42 °C for 45 s and placing it on ice for 2 min. Cells were then added to super optimal culture (SOC) medium (Sigma, USA) and incubated in a shaking incubator for 1–1.5 h at 37 °C. Aliquots (100–150 µL) of the transformed cultures were streaked onto selective Luria–Bertani (LB) agar plates containing 10 µg/mL ampicillin (Merck, USA) and incubated overnight at 37 °C. Transformed white colonies were selected and added into 5 mL LB medium containing ampicillin, in triplicate, and cultured for no more than 16 h in a shaking incubator at 37 °C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen, Germany) according to manufacturer's instructions. Plasmid concentration and purity were assessed using a Nanodrop 1000 spectrophotometer (Thermoscientific, USA).

Subcloning into expression vector, pLVX-EF1a-IRES Puro

Restriction enzyme digestion with BamHI and XbaI (NEB, England) was performed on both the IDT

vector containing the *KMT2A/MLLT1* fusion gene and the expression vector pLVX-EF1a-IRES Puro (Clontech, TAKARA), which carries multiple cloning sites, according to manufacturer's instructions.

Following digestion, gel electrophoresis was performed on 1% of agarose at 70 V and 200 mA for 1 h, and the digested bands were visualized on a transilluminator (GeneDireX Inc, USA). The band of interest (1683 bp) was excised from the gel and purified using the QIAquick gel extraction kit (Qiagen, Germany).

Ligation was performed with T4 DNA ligase enzyme (Promega, Korea), which joins the 5´-phosphate and 3´-hydroxyl groups of adjacent nucleotides in either cohesive-ended or blunt-ended configurations, according to manufacturer's protocol. The molar ratio of vector to insert DNA was maintained at least 1:3. Given that the size of pLVX-EF1a-IRES-Puro vector was 8.872 Kb and KMT2A/MLLT1 insert was 1.863 Kb, and vector DNA was kept at approximately 100 ng, the insert amount was calculated using the formula: ng vector/ng inserts (concentration x volume insert) = molar ratio (3/1) × (kb size vector/kb size insert).

T4 DNA ligase (1 U) was added to the buffer, and the reaction was incubated overnight in a chiller (0–4 °C). Various optimization steps were carried out, including adjustments to DNA concentration, vector:insert ratio, enzyme concentration, buffer and ATP levels, incubation conditions, ligation enhancers and, heat activation. The best results were achieved with 1:3 molar ratio [22].

The following day, bacterial transformation, plasmid extraction, restriction enzyme digestion, and gel electrophoresis were repeated to confirm successful ligation. Successful subcloning was validated by the presence of the expected band sizes of the gene of interest and expression vector (Supplementary Figure S2).

Restriction enzyme digestion was further performed to confirm the correct insertion and orientation of the newly generated recombinant plasmid DNA (Table 1).

Transfection of KMT2A/MLLT1 vector into PBMC

Transfection was performed using lipofection reagents (Lipofectamine 2000 and LTX reagent; Thermo Fisher, USA). One day before transfection, each well of a 12-well culture plate was seeded with 200,000 PBMCs in 1 mL of growth medium consisting of RPMI 1640, 10% fetal bovine serum, gentamicin (2 µg/mL), amphotericin B (1.25 µg/mL) and penicillin–streptomycin (100 U/mL) (all from Gibco, Invitrogen, USA), and incubated overnight at 5% CO_2, 37 °C.

Table 1 Restriction enzyme digestion was also performed to confirm the correct insertion and orientation of a newly generated recombinant plasmid DNA

No	Plasmid	Restriction enzyme	Restriction enzyme
1	MLL/MLLT1 (insert)	BamHI	Xbal
2	pLVX-EF1a-IRES-Puro (vector)	BamHI	Xbal

Transfection was first tested with the p65-mNeonGreen GFP positive control. Optimization was performed using this plasmid in both U937 monocytes cell lines and PBMCs. As recommended by the manufacturer, 2 µg of plasmid was used to optimize both cell types. The optimized conditions were then applied for transfection of the *KMT2A/MLLT1* vector into PBMCs.

Transfection reagent was prepared immediately before use. Briefly, plasmid DNA (2 µg) was diluted in serum-free RPMI (100 µL), mixed with 5 µL of each transfection reagent, and incubated for 5 min at room temperature. Then, 100 µL of the mixture was added dropwise to each well, except control wells, which received only the transfection reagent. Plates were gently rocked and then incubated at 37 °C in 5% CO_2 for 7 days with the p65-mNeonGreen GFP construct and up to 10 days with the *KMT2A/MLLT1* vector.

Flow cytometry analysis

GFP-positive cells were excited at 488 nm and detected at the 530 nm channel (FL1 detector) on a FACSCanto flow cytometer (BD, USA). Induced cells were characterized by flow cytometry using surface staining for B-cell leukemia-associated markers including monoclonal antibodies CD19-PE, CD34-PE-Cy7, CD45-PerCP-Cy5.5 and CD38-APC-H7 (all from BD Biosciences, USA). Expression of the KMT2A/MLLT1 fusion protein was detected by intracellular staining with polyclonal antibody MLL/KMT2A (Novus, USA), according to standard staining protocols.

Cell viability was assessed on day 10 using FVS 780 dye (FITC). Transfected and untransfected cells were harvested on days 3, 7 and 10. For intracellular staining, cells were fixed with Fix buffer/paraformaldehyde (PFA) (BD, Biosciences, USA) followed by permeabilization with Perm/Wash buffer (BD, Biosciences, USA). Cells were incubated with 1 µL primary polyclonal antibody for 45 min at 4 °C in the dark, followed incubation with 1 µL of FITC-conjugated goat anti-rabbit secondary antibody (Novus, USA).

Data acquisition was performed on FACSCanto flow cytometer (BD, USA). Cells were gated within the lymphoid population at forward scatter values of 50–100 to acquire a total of 10,000 events. The mean ± SD number of events acquired in the MLL + CD45 + gate on days 3, 7 and 10 was 2035 ± 1132; 373 ± 149; and 364 ± 323, respectively. Data analysis was performed using FACSDIVA software.

Statistical analysis

Statistical comparisons were performed between untransfected and transfected cells using the Mann–Whitney U test (Graphpad Prism, version 10). Results are presented as mean ± standard deviation (SD). A significance level of $p < 0.05$ was considered statistically significant.

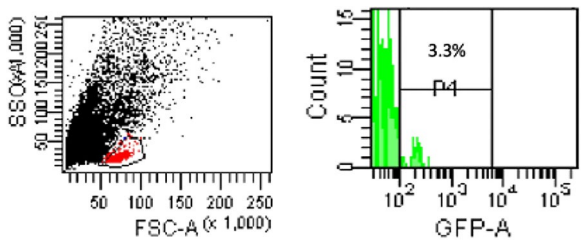

Fig. 1 Flow cytometry images of peripheral blood mononuclear cells (PBMC) transfected with the p65 mNeon GFP vector and showing successful expression of GFP (P4) on Day 7. Lipofection was used to transfect 200,000 PBMC in growth media for up to Day 7

Results

Transfection with the p65-mNeonGreen GFP plasmid was successfully detected by Day 7 (Fig. 1). The same procedure was then used to transfect the *KMT2A/MLLT1* vector into PBMCs collected from three volunteers. Three replicates were performed for each sample, resulting in nine datasets. A representative flow cytometry analysis is shown in Fig. 2. Lymphocyte-gated cells were CD45 positive (CD45+). Transfection with the *KMT2A/MLLT1* vector induced expression of MLL+in these cells, which was detectable in the P4 gate (right panel). The mean ± SD percentage of MLL+CD45+cells was significantly higher in transfected compared with untransfected cells at day 3 (71.4% vs. 1.7%, $p < 0.0001$), day 7 (42.7% vs. 0.1%, $p < 0.0001$) and day 10 (43.5% vs. 0.7%, $p < 0.0001$) (Fig. 3A). By contrast, the mean fluorescence intensity of CD45, CD34 and CD38 did not differ significantly between transfected cells and untransfected cells (Fig. 3).

This study focused on the induction of leukemia-like cells particularly of B-cell phenotype, since T-cell acute leukemias are rare. Interestingly, by day 10 a significantly higher percentage of B cells (35.4 ± 32.0, $p < 0.001$) was observed among MLL+cells that were also CD45+CD38+($p < 0.0039$) (Fig. 3B). In contrast,

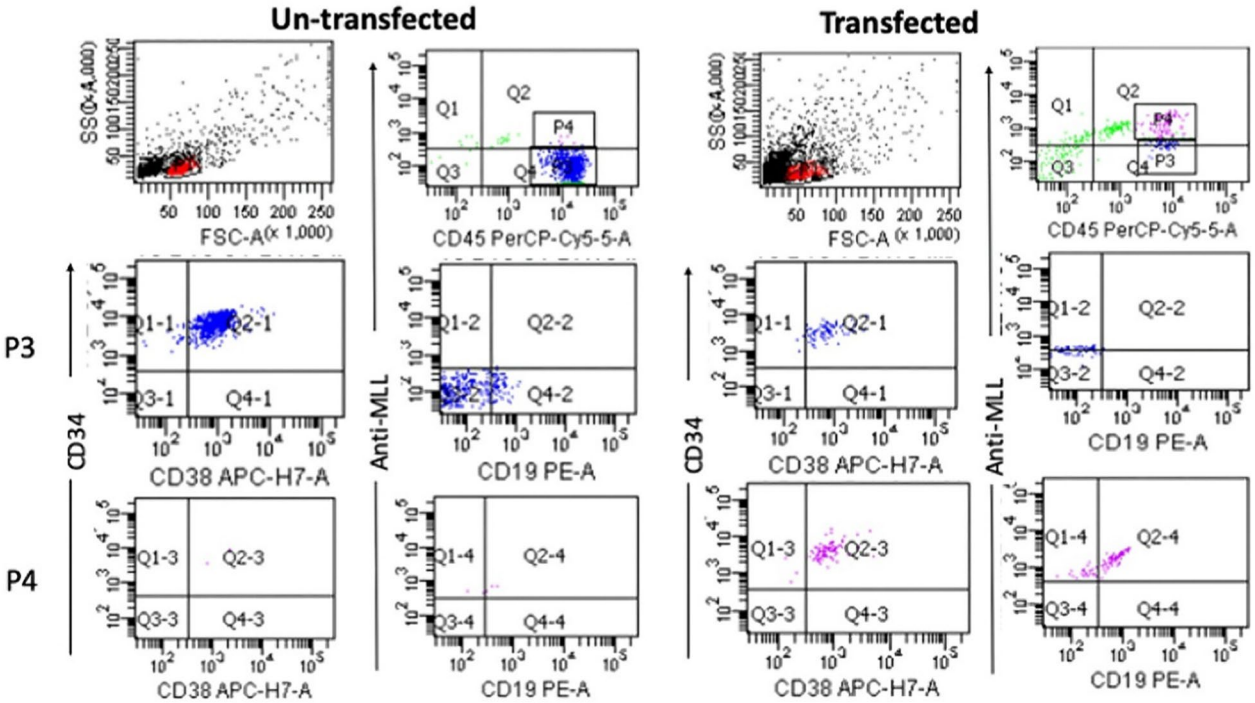

Fig. 2 Representative flow cytometry plots on PBMC un-transfected and transfected with MLL/MLLT1 fusion gene on day 10. The lymphocyte population (low FSC and low SSC) was first gated and then displayed for expression of MLL+/CD45+and subsequently observed for expression of CD34/CD38/CD19. While CD34 appears to be expressed, this is low compared to expression levels found in acute lymphoid and myeloid leukemia cases

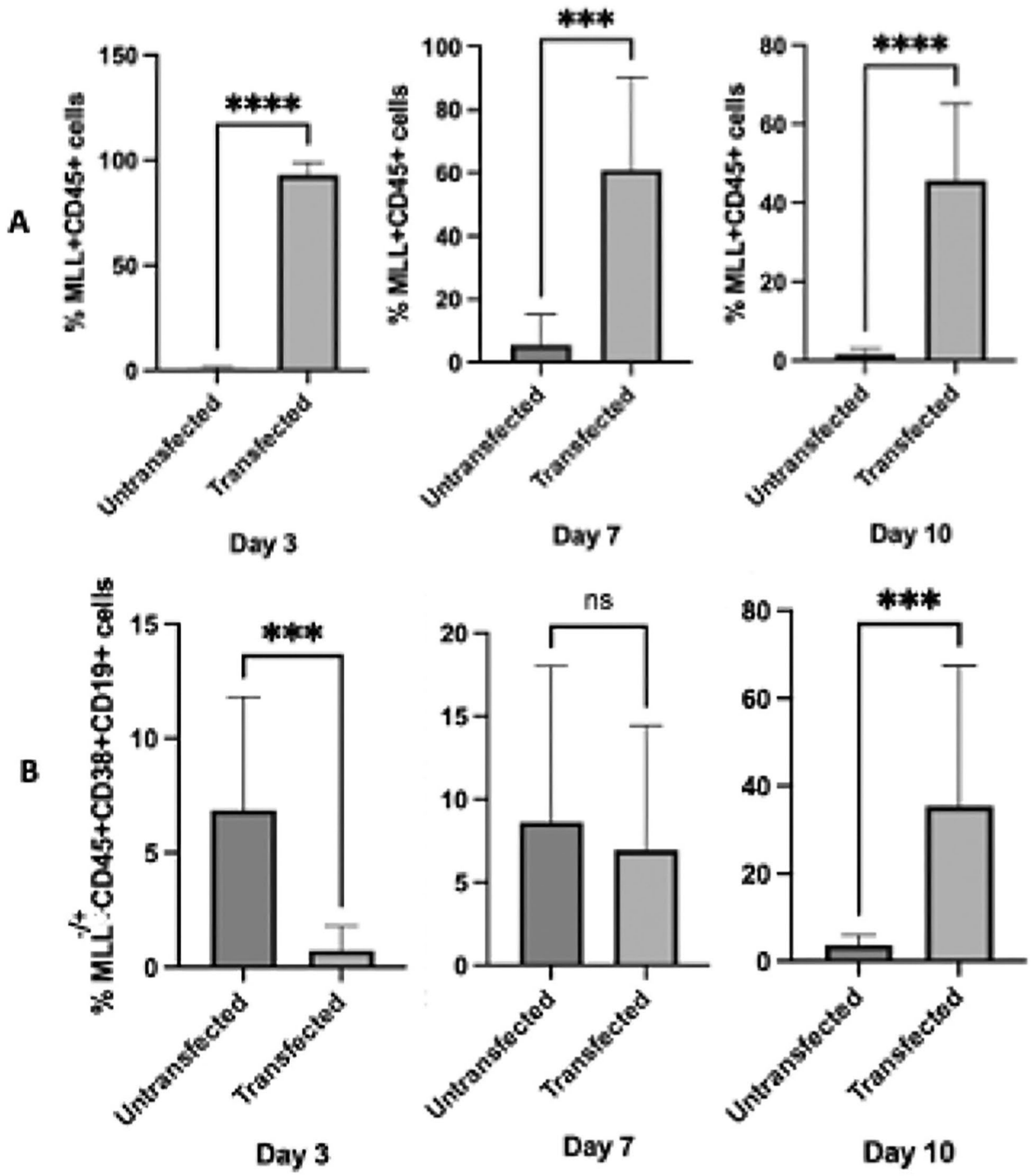

Fig. 3 Percentages of **A** MLL + CD45 + in un-transfected (P3) and transfected (P4) PBMC and **B**CD45 + CD38 + CD19 + in un-transfected (P3) and transfected (P4) PBMC with *KMT2A/MLLT1* vector at Day 3, Day 7 and Day 10 (N = 3, 9 experiments). Figure 3A Mean ± SD percentage of transfected and untransfected cells MLL + CD45 + cells after day 3 (71.4% v 1.7%, $p < 0.0001$), day 7 (42.7% v 0.1%, $p < 0.0001$) and day 10 of (43.5 v 0.7%, $p < 0.0001$). Figure 3B B cells (35.4 ± 32.0, $p < 0.001$) were observed among MLL + cells that were also CD45 + CD38 + on day 10 with $p < 0.0039$. This was in contrast to loss of B cells (3.7 ± 2.4) in cultures of un-transfected cells on day 3 with $p < 0.0078$. The high standard deviation was due to variation between individuals. ns–$p > 0.05$; ***$p < 0.001$; ****$p < 0.0001$

cultures of untransfected cells showed a loss of B cells (3.7 ± 2.4, $p < 0.0078$) (Fig. 3B).

Discussion

Acute lymphoblastic leukemia (ALL) is a malignancy of lymphoid progenitor cells that blocks early differentiation. It occurs in both children and adults, with peak incidence between age 1 to 4 years [23], and approximately 75% of cases occurring in pediatric patients. More than 240 000 new cases of childhood acute leukemia are diagnosed each year [24]. Chronic lymphomas and leukemias, including chronic lymphocytic leukemia (CLL), on the other hand, affect mature cells [25].

Chromosomal translocation is a major cause of leukemia. Approximately 75% of childhood ALL cases harbor recurrent genetic abnormalities such as aneuploidy and structural chromosomal arrangements, including translocations [26].

The *KMT2A* fusion gene has been used extensively to generate animal models of acute leukemia and has been instrumental in elucidating disease mechanisms and identifying therapeutic targets.

However, because *KMT2A* can fuse with many partner genes, the diseases generated are often unpredictable and may develop only after a long latency period [14]. The microenvironment also plays a critical role in cancer development, providing support from stromal cells and eradication by the immune system, that are not observable in irradiated or immunodeficient mice. Furthermore, *KMT2A/MLLT1*-induced AML cells transferred into non-conditioned wildtype hosts are unable to propagate [27].

Peripheral blood mononuclear cells (PBMCs) consist mainly of lymphocytes and monocytes and represent an important source of B cells. These cells spontaneously produce cytokines at low levels early in cell culture [28]. Nevertheless, they provide a microenvironment conducive to short-term cell growth, particularly for B cells. PBMCs have also been a convenient source of primary cells for studies ranging from inflammatory responses to vaccine research, and they are commonly used in the assessment of immunocompetence. Cryopreserved PBMC samples can retain at least 90% viability for more than 12 years [29], and unstimulated PBMC cultures in standard medium can maintain over 60% viability after 10 days in vitro [30].

In this study, adult PBMCs were transfected with the *KMT2A/MLLT1* fusion gene. This approach differs from most previous studies, which primarily aimed to recapitulate the original disease in early progenitor cells. Since *KMT2A*-rearrangements are also detected in B cell lymphomas [11], which are mature-cell malignancies, the model generated here may be particularly suitable for studying advanced forms of B-cell cancers. Thus, PBMCs provide a convenient and ethically favorable source of cells for transformation.

The majority of lymphoma/lymphoblastic leukemias are of B cell origin. Although KMT2A rearrangements are associated with AML, ALL and even mixed-lineage leukemia, experimental induction of leukemia with *KMT2A* fusion genes shows lineage-specific predispositions. Barabé et al. (2007) reported that B-cell acute lymphoblastic leukemia (ALL) was most frequently induced with *KMT2A/MLLT1* and *MLL-AF9* [31]. In murine models, *MLL-AF9* generated AML from mouse hematopoietic stem and progenitor cells (HSPCs) but pro-B ALL from human HSPCs [32]. While *MLL-AF9* expressed in human CD34 + HSPCs induced both AML and ALL in mice, *KMT2A/MLLT1* gave rise exclusively to the ALL subtype [17]. As our aim was to generate a B-cell associated malignancy, the *KMT2A/MLLT1* construct from [17] was selected.

Using the TALENs method, Buechele et al. (2015) successfully transfected approximately 20% of K562 cells and lower percentages of CD34 + HSPCs with *KMT2A/MLLT1* [17]. In this study, GFP was expressed in 3.3% of PBMCs within the lymphocyte compartment. Toxicity and transfection efficiency are generally positively correlated [32]. By day 10, approximately 45% of PBMCs were CD45 + *KMT2A/MLLT1* + . Reimer et al. (2017) reported the formation of true t(11;19)/MLL-AF9 translocations both in vitro and in vivo using CRISPR-Cas9; however, full transformation was not observed in liquid culture or methylcellulose assays with CD34 + hematopoietic stem progenitors cells (HSPCs) [33].

In control experiments, untransfected cells treated with Lipofectamine but without the *KMT2A/MLLT1* vector remained MLL negative. Approximately 7% of B cells were detected in early cultures, consistent with the expected 5–10% found in healthy PBMCs. Over time, the proportion of B cells declined to nearly zero by day 10.

Transduction or transfection is defined as a non-replicative, dead-end process that enables delivery of genetic information into specific cell [34]. In this study, transient transfection was used [35]. These cells were not expected to persist long term, as the KMT2A*/MLLT1* vector would be diluted with each cell division. Alternative approaches, such as mechanical transfection or electroporation, may also be applied to primary cells. Electroporation exposes cells to an electric field of sufficient amplitude to facilitate plasmid DNA entry [36]. Factors such as cell density, media culture condition and DNA plasmid concentration must be carefully optimized. For instance, serum-free medium in combination with RPMI has been shown to support viable and proliferating B cells for up to six days in culture [37]. Liposomes or nanoparticles may further

enhance DNA delivery into primary B cells. Liposomes are nano-vesicles enveloped by lipid bilayers, offer promising biological features due to their resemblance to natural biomembranes [38].

By addressing these methodological factors and exploring alternative delivery systems, researchers may improve transfection efficiency in primary B cells. Nonetheless, interindividual variability remains a significant limitation in generating leukemia-like cells, as genetic background, cellular responses, and disease progression differ among donors and can affect the reproducibility and accuracy of the model. Future studies should incorporate diverse donor pools to capture this variability and apply standardized protocols to minimize experimental inconsistencies. Moreover, advanced genomic and proteomic analyses could help identify sources of variability, thereby supporting the development of more robust and representative leukemia models.

Electroporation and liposomal delivery systems are promising approaches for efficient gene and drug transfer into leukemia-like cells because they enhance transfection efficiency while reducing cytotoxicity. Electroporation applies brief electrical pulses to create transient pores in cell membranes, enabling direct entry nucleic acids or drugs. This approach is particularly effective for hard-to-transfect leukemia cells. Liposomal systems encapsulate therapeutic agents within lipid bilayers, facilitating targeted delivery and improved cellular uptake while minimizing off-target effects. Future experimental directions include optimizing electroporation parameters (e.g., voltage, pulse duration) to improve specificity and viability, and designing next-generation liposomes with surface modifications for targeted delivery to leukemia cells. Combining these techniques with precision medicine approaches and high-throughput screening could lead to more personalized and effective therapeutic strategies [39].

In contrast, within transfected MLL + samples, a population of B cells emerged and reached its highest proportion by day 10. This finding is consistent with previous studies showing that *KMT2A/MLLT1* induces B cells with altered growth potential [17, 31]. Another study that used primary human B cells modelled high-grade lymphoma by applying CRISPR/Cas9 to express *CCND3*, *BCL6*, or *IL21* in human germinal center B cells, which also required FDC-like feeder cells for long-term culture [40]. CRISPR/Cas 9 has also been used in vivo to deliver BCR-ABL, t(9;22), effectively slowing progression of Philadelphia-positive ALL with the T315I mutation [41].

B-cell acute lymphoblastic leukemia is typically characterized by high CD34 expression, reduced CD45, and the presence of CD19 and CD38. These markers were detected on the abnormal B-cell population in this study but showed no change from baseline, suggesting that progenitor features were not induced. By contrast, B-cell lymphomas variably express markers such as CD10, BCL-2 and CD5 [42], which were not examined here.

This leukemia-like cell model enables the use of samples from individual selected on specific criteria, such as genetic background, including variation in the highly polymorphic HLA complex, to study interactions with the microenvironment without rejection issues.

Future research may explore inducing pluripotent stem cell features before leukemia induction to generate cells resembling the immature phenotype of acute leukemias. However, developing in vitro leukemia models remains challenging because of the complexity of bone marrow niches, leukemia-associated stem cells and related factors. Current preclinical approaches include 2D in vitro culture models and, more recently, 3D culture models of acute lymphoblastic leukemia using gelatin-based matrices [43]. These advances may facilitate studies of leukemia pathogenesis, biomarker discovery and drugs development. The proposed model offers a cost- and time-efficient approach to generating leukemia-like cells, albeit transiently. Because transient transfection does not integrate nucleic acids into the host cell genome, it is well suited for in vitro cytotoxicity screening that requires short-term assays such as the MTT assay (3 day drug exposure). This system is therefore valuable for preclinical drug screening, testing synergistic combinations, and identifying biomarkers of drug response and resistance [44].

The development of cancer therapeutics remains a major hurdle in leukaemia, as responses may vary across populations and individuals. The ability to rapidly manipulate and transform cells could accelerate clinical decision-making and improve patient management.

Conclusion

This simple, rapid, and relevant method of overexpressing *KMT2A/MLLT1* in peripheral blood mononuclear cells successfully generated B cells with abnormal growth potential. These cells may serve as a practical source of 'leukemia-like' cells for further study.

Limitations of the study

Several limitations should be acknowledged in this study. The samples size was small; only three subjects from both sexes were included, which may have contributed to variability between individuals. Future studies should involve larger, more homogeneous cohorts stratified by sex, race, and age to enhance reproducibility and generalizability. B cells could also be isolated to increase yield. In this study, peripheral blood was used, which contains primary suspension cells that are inherently difficult to transfect.

Future work should consider mechanical methods such as electroporation to improve transfection efficiency and reprogramming of these cells.

Abbreviations

ABL	Abelson
AFF1	ALF transcription elongation factor 1
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BCR	Breakpoint cluster region
CD	Cluster of differentiation
CLL	Chronic lymphocytic leukemia
CRISPR	Clustered regularly interspaced short palindromic repeats
DLBCL	Diffuse large B cell lymphoma
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E2A	Transcription factor 3 (TCF3)
ETV6	ETS variant transcription factor 6
FITC	Fluorescein isothiocyanate
FVS	Fixable viability stain
GFP	Green fluorescent protein
HLA	Human leukocytes antigen
HSPC	Hematopoietic stem and progenitor cells
IDT	Integrated DNA technologies
KMT2A	Histone-lysine N-methyltransferase 2A
KMT2A-r	*KMT2A* rearrangement
MREC	National Medical Research Ethics Committee Registry
MLL	Mixed lineage leukemia gene
NGS	Next gene sequencing
NIH	National Institute of Health
PBMC	Peripheral blood mononuclear cells
PFA	Paraformaldehyde
RNA	Ribonucleic acid
RUNX	Runt-related transcription factor 1
SD	Standard deviation
TALENS	Transcription activator-like effector nucleases
TPG	Translocation partner gene
WHO	World Health Organisation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-025-00786-5.

Additional file 1.

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Author contributions
Conceptualization, design, analysis & writing, M.A., design, data collection processing, analysis or interpretation, literature search & writing, S.Z.I, data collection processing, V.P, data collection processing, A.M.A, surgical and medical practice, S.T.S, Conceptualization L.M.I & F.N.

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Data availability
Supplementary 1.

Declarations

Ethics approval and consent to participate
Approvals to conduct the study were obtained from the Ethics Committee for Research Involving Human Subjects (JKEUPM), Universiti Putra Malaysia and National Medical Research Ethics Committee Registry (MREC), Ministry of Health Malaysia (NMRR-17-2697-38131).

Consent for publication
All authors read and approved the final manuscript. And agree to the publication of the manuscript.

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The authors declare no competing interests.

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References

1. Alaggio R (2022) The 5th edition of the World Health Organization classification of hematolymphoid tumours: lymphoid neoplasms. Leukemia 36(7):1720–1748. https://doi.org/10.1038/s41375-022-01620-2
2. Cazzola M (2016) Introduction to a review series: the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues. Blood 19(20):2361–2364. https://doi.org/10.1182/blood-2016-03-657379
3. Huang J, Chan SC, Ngai CH, Lok V, Zhang L, Lucero-Prisno DE, Xu W, Zheng ZJ, Elcarte E, Withers M, Wong MCS (2022) Disease burden, risk factors, and trends of leukaemia: a global analysis. Front Oncol 12(July):1–8. https://doi.org/10.3389/fonc.2022.904292
4. Vardiman JW (2002) The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 100(7):2292–302. https://doi.org/10.1182/blood-2002-04-1199
5. Meyer C (2023) The KMT2A recombinome of acute leukemias in 2023. Leukemia 37(5):988–1005. https://doi.org/10.1038/s41375-023-01877-1
6. Arber DA (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127(20):2391–2405. https://doi.org/10.1182/blood-2016-03-643544
7. Britten O (2019) MLL-rearranged acute leukemia with t(4;11)(q21;q23)-current treatment options. Is there a role for CAR-T cell therapy? Cells 8(11):1–22. https://doi.org/10.3390/cells8111341
8. Behm FG (1996) Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood. 87(7):2870–7
9. Qiu Ky (2022) Prognostic value and outcome for acute lymphocytic leukemia in children with MLL rearrangement: a case-control study. BMC Cancer 22:1257. https://doi.org/10.1186/s12885-022-10378-w
10. Goldman JW, Mody RJ (2024) Further validation of poor prognosis for pediatric *KMT2A*-rearranged leukemia and the need for rapid integration of targeted therapies for these patients. Transl Pediatr 13(2):370–375. https://doi.org/10.21037/tp-23-567
11. Kato S (2021) *KMT2A*-rearranged diffuse large B-cell lymphoma in a child: a case report and molecular characterization. Pediatr Hematol Oncol. https://doi.org/10.1080/08880018.2020.1838013
12. Milne TA (2017) Mouse models of MLL leukemia: recapitulating the human disease. Blood 20(16):2217–2223. https://doi.org/10.1182/blood-2016-10-691428
13. Reilly MM, Rossor AM (2020) Humans: the ultimate animal models. J Neurol Neurosurg Psychiatry 91(11):1132–1136. https://doi.org/10.1136/jnnp-2020-323016
14. Schwaller J (2020) Learning from mouse models of MLL fusion gene-driven acute leukemia. Biochimica et Biophysica Acta Gene Regul Mech 1863(8):194550. https://doi.org/10.1016/j.bbagrm.2020.194550
15. Petropoulos C (1997) Retroviral taxonomy, protein structures, sequences, and genetic maps in retroviruses. In: Coffin JM, Hughes SH, Varmus HE (eds). Cold Spring Harbor (NY), Cold Spring Harbor Laboratory Press

16. Corral J, Lavenir I, Impey H (1996) An Mll-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell. 85(6):853–61
17. Buechele C, Breese EH, Schneidawind D (2018) MLL leukemia induction by genome editing of human CD34 1 hematopoietic cells. Lymphoid Neoplasia. 126(14):1683–1695. https://doi.org/10.1182/blood-2015-05-646398
18. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32(4):347–355. https://doi.org/10.1038/nbt.2842
19. Perez-Chacon G, Vincent-Fabert C, Zapata JM (2021) Editorial: Mouse models of B cell malignancies. Front Immunol 12:789901. https://doi.org/10.3389/fimmu.2021.789901
20. Kaur G, Dufour JM (2012) Cell lines. Spermatogenesis 2(1):1–5. https://doi.org/10.4161/spmg.19885
21. Isidro-Hernández M, Alemán-Arteaga S, Casado-García A (2022) Childhood B-cell preleukemia mouse modeling. Int J Mol Sci 23(14):1–14. https://doi.org/10.3390/ijms23147562
22. Alexander RC, Johnson AK, Thorpe JA, Gevedon T, Testa SM (2003) Canonical nucleosides can be utilized by T4 DNA ligase as universal template bases at ligation junctions. Nucleic Acids Res 31(12):3208–3216. https://doi.org/10.1093/nar/gkg415
23. Malard F, Mohty M (2020) Acute lymphoblastic leukaemia. Lancet 395(10230):1146–1162. https://doi.org/10.1016/s0140-6736(19)33018-1
24. Jaime-pérez JC, García-arellano G, Herrera-garza, (2018) Transfusion and cell therapy original article revisiting the complete blood count and clinical findings at diagnosis of childhood acute lymphoblastic leukemia: 10-year experience at a single center. Hematol Transfus Cell Ther 41(1):57–61. https://doi.org/10.1016/j.htct.2018.05.010
25. Dong Y, Shi O, Zeng Q (2010) Leukemia incidence trends at the global, regional, and national level between 1990 and 2017. Exp Hematol Oncol 9(1):1–11. https://doi.org/10.1186/s40164-020-00170-6
26. Woo J, Baumann A, Arguello V (2014) Recent advancements of flow cytometry: new applications in hematology and oncology. Expert Rev Mol Diagn 14(1):67–81. https://doi.org/10.1586/14737159.2014.862153
27. Nakata J, Nakano K, Okumura A (2014) In vivo eradication of MLL/ENL leukemia cells by NK cells in the absence of adaptive immunity. Leukemia 28(6):1316–1325. https://doi.org/10.1038/leu.2013.374
28. Friberg D, Bryant J, Shannon W (1994) In vitro cytokine production by normal human peripheral blood mononuclear cells as a measure of immunocompetence or the state of activation. Clin Diagn Lab Immunol. 1(3):261–8
29. Kleeberger CA, Lyles RH, Margolick JB (1999) Viability and recovery of peripheral blood mononuclear cells cryopreserved for up to 12 years in a multicenter study. Clin Diagn Lab Immunol 6(1):14–19. https://doi.org/10.1128/cdli.6.1.14-19.1999
30. Gong S, Fajar P, De Vries-Idema J (2023) Comparison of media for a human peripheral blood mononuclear cell-based in vitro vaccine evaluation system. Clin Exp Vaccine Res 12(4):328–336. https://doi.org/10.7774/cevr.2023.12.4.328
31. Barabé F, Kennedy JA, Hope KJ (2007) Modeling the initiation and progression of human acute leukemia in mice. Science 27(5824):600–604. https://doi.org/10.1126/science.1139851
32. Lin S, Luo RT, Ptasinska A (2016) Instructive role of MLL-fusion proteins revealed by a model of t(4;11) pro-B acute lymphoblastic leukemia. Cancer Cell 14(30):737–749. https://doi.org/10.1016/j.ccell.2016.10.008
33. Reimer J (2017) CRISPR-Cas9-induced t(11;19)/MLL-ENL translocations initiate leukemia in human hematopoietic progenitor cells in vivo. Haematologica 102(9):1558–1566. https://doi.org/10.3324/haematol.2017.164046
34. Vannucci L, Lai M, Chiuppesi F (2013) Viral vectors: a look back and ahead on gene. New Microbiol 2013(36):1–22
35. Kim TK, Eberwine JH (2010) Mammalian cell transfection: the present and the future. Anal Bioanal Chem 397(8):3173–3178. https://doi.org/10.1007/s00216-010-3821-6
36. Napotnik BT, Polajžer T, Miklavčič D (2021) Cell death due to electroporation—a review. Bioelectrochemistry 141:107871. https://doi.org/10.1016/j.bioelechem.2021.107871
37. Cochrane S, Saib O, Sheffield D (2024) Use of serum-free media for peripheral blood mononuclear cell culture and the impact on T and B cell readouts. Front Toxicol 6(November):1–13. https://doi.org/10.3389/ftox.2024.1462688
38. Nsairat H (2023) Recent advances in using liposomes for delivery of nucleic acid-based therapeutics. Open Nano 11(January):100132. https://doi.org/10.1016/j.onano.2023.100132
39. Chong ZX, Yeap SK, Ho WY (2021) Transfection types, methods and strategies: a technical review. PeerJ 9:1–37. https://doi.org/10.7717/peerj.11165
40. Caeser R, Di Re M, Krupka JA (2019) Genetic modification of primary human B cells to model high-grade lymphoma. Nat Commun 4:4543. https://doi.org/10.1038/s41467-019-12494-x
41. Tan Y-T (2020) Letters to the editor. Haematologica 105(5):1–5. https://doi.org/10.3810/psm.1999.02.687
42. Cho J (2022) Basic immunohistochemistry for lymphoma diagnosis. Blood Res. 57(S1):55–61. https://doi.org/10.5045/br.2022.2022037
43. Mattioda C, Voena C, Ciardelli G, Mattu C (2025) In vitro 3D models of haematological malignancies: current trends and the road ahead? Cells 14(1):1–24. https://doi.org/10.3390/cells14010038
44. Cucchi DGJ, Groen RWJ, Janssen JJWM, Cloos J (2020) Ex vivo cultures and drug testing of primary acute myeloid leukemia samples: Current techniques and implications for experimental design and outcome. Drug Resist Updates 53(July):100730. https://doi.org/10.1016/j.drup.2020.100730

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