

Dietary intervention targets hierarchical metabolic dependencies in human acute myeloid leukemia

Maria Lastra Cagigas

maria.lastracagigas@sydney.edu.au

The University of Sydney <https://orcid.org/0000-0001-5464-2349>

Gayathiri Rajakumar

The University of Sydney

Tiana Pelaia

The University of Sydney

Rachael Hayward

The University of Sydney

Yue Cao

The University of Sydney

Dawei Zheng

Westmead Institute for Medical Research

Lake Ee Quek

The University of Sydney <https://orcid.org/0000-0002-9313-8740>

Andrius Masedunskas

The University of Sydney

Lewin Small

<https://orcid.org/0000-0002-9767-9464>

Kellie Wise

Children's Cancer Institute at Minderoo Children's Comprehensive Cancer Centre

Kristen Skarratt

The University of Sydney

Alireza Ardjmand

Nepean Hospital <https://orcid.org/0009-0002-8023-9689>

Moritz Warmbrunn

The University of Sydney

Raaj Biswas

Charles Perkins Center, Faculty of Medicine and Health, The University of Sydney

<https://orcid.org/0000-0003-4294-6323>

Lachlin Vaughan

Westmead Hospital

Luciano Martelotto

Waters Biosciences

Jean Yang

The University of Sydney <https://orcid.org/0000-0002-5271-2603>

Stephen Fuller

The University of Sydney

Luigi Fontana

Charles Perkins Center, University of Sydney, Sydney <https://orcid.org/0000-0001-8500-5537>

Article

Keywords: Cell metabolism, leukemia metabolism, carbohydrate restriction, ketogenic diet

Posted Date: July 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9930778/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Abstract

Acute myeloid leukemia (AML) is sustained by metabolic plasticity, enabling leukemic cells to adapt across cellular states and withstand therapeutic stress. Whether systemic manipulation of nutrient availability can expose metabolic dependencies in human leukemia remains unknown.

We conducted a randomized metabolic intervention in patients with newly diagnosed AML undergoing induction chemotherapy to determine whether short-term carbohydrate restriction could reprogram leukemic metabolism *in vivo*. Patients received an 8–12-day ketogenic diet (< 10% carbohydrate) during hospitalization. The study was underpowered for its primary clinical endpoint (infection rates) because of recruitment limitations; analyses therefore focused on pre-specified secondary and mechanistic outcomes.

Integrating longitudinal plasma metabolomics, proteomics (5,751 proteins), and single-cell transcriptomics (5,101 genes) across more than two million primary cells from over 100 patient samples, we mapped the systemic and cell-state-specific consequences of dietary intervention in AML. Ketogenic diet reduced glucose and insulin exposure while increasing circulating ketones, suppressing ribosome biogenesis, nucleotide synthesis, and anabolic metabolism in proliferative leukemic blasts. These changes were accompanied by adaptive engagement of fatty-acid-supported mitochondrial metabolism. In contrast, stem- and progenitor-like populations implicated in relapse failed to mount comparable adaptive responses, instead exhibiting broad metabolic and transcriptional repression while preserving stemness programs.

Together, these findings identify hierarchical metabolic dependencies in human AML and demonstrate that dietary intervention can functionally remodel leukemic cell states in patients. Our study establishes organism-level metabolism as a therapeutic framework for targeting therapy-persistent leukemia populations.

PLAIN MESSAGE

Changing what patients eat may stress cancer cells by limiting their fuel supply. This could be especially important in acute myeloid leukemia (AML), where leukemic cells switch between energy sources to survive treatment. However, this has not been tested in patients. We show that a ketogenic diet is feasible during intensive chemotherapy and rapidly changes the body's metabolism, lowering key growth-supporting nutrients. While mature leukemia cells adapt by using lipid-based energy, immature stem-like cells fail to respond in the same way. This reveals a metabolic constraint in leukemia cells linked to relapses that could be targeted with future combination therapies.

INTRODUCTION

Acute myeloid leukemia (AML) is a highly lethal hematologic malignancy in which long-term survival remains poor despite advances in genomic classification and targeted therapies. (1, 2) This limited

therapeutic durability reflects, in part, the extraordinary metabolic plasticity of leukemic cells, which enables survival under therapeutic stress and contributes to disease persistence and relapse. (3–7) Defining metabolic dependencies that are both biologically fundamental and therapeutically actionable therefore represents a major challenge in AML.

AML is sustained by metabolically distinct cellular compartments. Rapidly proliferating myeloblasts exhibit high glycolytic and biosynthetic demand to support nucleotide synthesis, ribosome biogenesis, and protein translation, whereas rare leukemic stem cells (LSCs) depend predominantly on mitochondrial oxidative phosphorylation (OxPhos), supported in part by amino acid catabolism. This metabolic compartmentalization allows dynamic adaptation to nutrient availability and chemotherapy, rendering strategies targeting single pathways insufficient to eradicate disease. (3, 4, 6, 8–14)

The central role of biosynthetic and translational metabolism in AML (15, 16) has motivated therapeutic strategies targeting glycolysis, (17) ribosome formation, (18) and protein translation, (19) which suppress myeloblast proliferation and enhance chemotherapy efficacy. However, LSCs, which proliferate slowly and drive relapses, are relatively resistant to these approaches. (2, 3, 6, 14, 20–25) LSC metabolism can change upon therapy exposure (3, 23): in treatment-naïve AML, amino acid catabolism is known to sustain OxPhos, whereas following chemotherapy or at relapse, LSCs can shift toward a greater reliance on fatty acid oxidation (FAO), a rewiring that contributes to chemoresistance. (14, 20–22) These observations suggest that effective metabolic targeting requires approaches capable of constraining multiple leukemic states simultaneously. (3, 9)

Systemic modulation of nutrient availability offers a clinically feasible strategy to impose such broad metabolic pressure. (26–31) Carbohydrate-restricted ketogenic diets (KD) reduce circulating glucose and insulin, alter systemic amino acid availability, and shift whole-body metabolism toward lipid oxidation. (32–34) However, whether these systemic metabolic changes can directly reprogram the molecular state of human leukemia cells *in vivo* during chemotherapy remains unknown.

Here, we address this question in a randomized controlled trial of patients with newly diagnosed AML undergoing anthracycline–cytarabine induction therapy. By integrating longitudinal plasma metabolomics with deep proteomic and single-cell transcriptomic profiling of primary leukemic cells, we define the *in vivo* effects of ketogenic intervention on leukemia metabolism. Crucially, this approach enabled resolution of cell-state-specific responses that would be obscured in bulk leukemic analyses.

We find that ketogenic intervention imposes an energetic constraint on leukemia in a state-dependent manner, with distinct responses across leukemic populations. Together, these findings show that systemic nutrient manipulation can directly rewire leukemia in patients and reveal therapeutically actionable metabolic liabilities.

RESULTS

A ketogenic dietary intervention is feasible during induction chemotherapy and remodels systemic metabolism

In a randomized controlled trial of newly diagnosed AML receiving standard induction chemotherapy using the 7 + 3 regimen (7 days of cytarabine and 3 days of an anthracycline),(1) 134 patients were screened between October 2022 and December 2024 across two tertiary hospitals (Nepean and Westmead) in Sydney, Australia, and 21 were randomized in a 3:2 ratio (KD:control) to a KD (n = 13) or control hospital diet (n = 8). The CONOSRT diagram is provided in **Fig. S1**, and inclusion and exclusion criteria are presented in **Table S1**. One control participant was withdrawn prior to study initiation due to revision of diagnosis, leaving 20 evaluable participants with AML confirmed by standard diagnostic assessment, including flow cytometric evidence of aberrant myeloid blasts (> 20%) expressing myeloid markers (e.g. CD33⁺, CD117⁺, CD13⁺) (**Fig. S1**, Fig. 1A-C). Participants had a mean age of 49 ± 15 years, a mean BMI of 30.3 ± 9.0 kg/m² and were risk-stratified according to the 2022 European LeukemiaNet classification. All participants received standard induction chemotherapy, with targeted agents administered according to mutational or cytogenetic status (midostaurin for FLT3-mutant AML, n = 7; gemtuzumab ozogamicin for favorable-risk cytogenetics, n = 4) (**Table 1**). Three patients (15%) failed to achieve morphological remission after induction.

Participants randomized to KD received hospital-prepared meals providing ~ 80% of total energy from fat. The intervention was initiated 2–3 days prior to chemotherapy and continued throughout the 7-day induction regimen, with a mean duration of 8.7 ± 2.3 days (Fig. 1A, 1D, **Table S2**, **Table S3**, **Table S4**). Ten of thirteen participants completed the full intervention, whereas three discontinued early because of dietary intolerance (**Figure S1**; **Table S4**), indicating that short-term KD is largely feasible during intensive inpatient chemotherapy.

KD induced rapid and robust systemic metabolic remodeling. Within 2–3 days, circulating levels of the ketone β -hydroxybutyrate (BHB) rose significantly from baseline levels in KD participants, while remaining negligible in controls (between-group $q = 0.0129$) (Fig. 1E), confirming nutritional ketosis. Even though glucose levels were similar immediately before treatment, KD markedly blunted the chemotherapy-associated hyperglycemic response observed in the participants (5.88 ± 0.68 mmol/L in KD vs 7.80 ± 2.74 mmol/L in control, between-group $q = 0.0044$) (Fig. 1F). Circulating insulin was also substantially reduced both before and during treatment (16.0 – 17.5 μ U/mL in KD vs 47.6 – 53.2 in control μ U/mL, between-group $q = 0.0188$ at Pre and $q = 0.0172$ during Chemo) (Fig. 1G), indicating attenuation of anabolic insulin signaling. These changes were reversible upon cessation of the intervention.

KD selectively altered circulating amino acid availability. Prior to chemotherapy, the glucogenic amino acids alanine and proline were significantly reduced in KD participants (-33% , $q = 0.0420$ and -16% , $q = 0.0006$, respectively) (Fig. 1H), indicating reduced availability of key gluconeogenic substrates. Given the

dependence of LSCs on proline,(23) this early depletion may limit a key substrate that supports leukemic maintenance.

During chemotherapy, circulating amino acids increased broadly in both groups, consistent with treatment-induced proteolysis, with significant increases in asparagine, lysine, methionine, phenylalanine, glutamine, tyrosine, and arginine detected in both groups (Fig. 1I). However, this increase was partially blunted in KD participants for a subset of glucogenic and serine–glycine–linked amino acids, including alanine, proline, threonine, and serine (Fig. 1H), suggesting partial suppression of chemotherapy-associated amino acid release. In contrast, branched-chain amino acids (BCAAs; leucine, isoleucine, and valine) increased to a similar extent in both groups during chemotherapy, with the KD group exhibiting an additional pre-treatment rise attributable to dietary intake (Fig. 1J).

Together, these data indicate that a short-term ketogenic dietary intervention rapidly remodels the systemic nutrient environment, reducing the availability of substrates implicated in leukemic cell growth and survival.

Deep proteomic profiling of primary leukemic cells defines a replication-stressed baseline during induction chemotherapy

To establish the molecular context in which dietary intervention operates, we first characterized the proteomic response of primary leukemic cells to standard 7 + 3 induction chemotherapy. Peripheral blood was collected longitudinally at baseline (n = 20), immediately prior to chemotherapy (n = 19), and during early treatment (n = 18; one withdrawal and one QC failure). Leukemic cells were enriched from peripheral blood mononuclear cells (PBMCs) using a CD33-targeting antibody, a robust and clinically validated AML surface marker (35), achieving > 99% purity and > 90% viability (Fig. 1A, Fig. 2A). Using a minimum of 500,000 viable cells per sample, untargeted LC–MS/MS proteomics detected 5,827 proteins, of which 5,751 were mapped to UniProt identifiers and annotated for subcellular localization. Quality control confirmed high technical reproducibility, with tight clustering in principal component and multidimensional scaling analyses and no outliers detected (**Fig. S2**).

Induction chemotherapy elicited widespread proteomic remodeling. Repeated-measures mixed-effects modelling identified 1,100 significantly altered proteins (402 upregulated, 698 downregulated; FDR < 5%) across both dietary groups (Fig. 2B, **Fig. S3**). Comparison with > 200,000 public datasets (IPA Qiagen) confirmed significant similarity with AML and monocytic cells exposed to cytarabine and anthracyclines (**Fig. S4**), validating the biological specificity of the observed responses. Proteomic alterations associated with anthracycline treatment are presented in Fig. 2C.

Downregulated proteins were strongly enriched for DNA replication, chromatin organization, and cell cycle progression (Fig. 2B, 2D, **Fig. S5**). Core components of the replication machinery, including the MCM helicase subunits MCM2, MCM3, and MCM5, the sliding clamp PCNA, DNA polymerase subunits, and the nucleotide synthesis enzyme RRM1, were significantly reduced. Chromatin organization proteins

(including CTCF) and mismatch repair factors (including MSH2 and MSH6) were also suppressed, consistent with impaired genome maintenance. Reduced abundance of MKI67 reflected decreased proliferative capacity, while broad downregulation of proteins involved in rRNA and mRNA processing, splicing, and transcriptional regulation indicated translational shutdown (Fig. 2B, 2D, **Fig. S5**).

In parallel, proteins associated with DNA damage response, checkpoint signaling, and tumor suppressor pathways were increased (Fig. 2B, 2D). Activation of TP53- and PTEN signaling, together with increased CDKN1A (p21) and CDKN2A (p16), indicated cell-cycle arrest. Proteomic alterations associated with TP53 activation are shown in Fig. 2E and **Fig. S6**. Increased MCL1 further suggested engagement of intrinsic apoptotic signaling, while elevated cytoskeletal regulators were consistent with structural remodeling during cell-cycle arrest (Fig. 2D).

Collectively, these data define the canonical *in vivo* response of AML cells to anthracycline–cytarabine induction chemotherapy, characterized by a replication-stressed leukemic state marked by suppressed DNA replication, checkpoint activation, proliferative arrest, and transcriptional shutdown. (36, 37) This proteomic landscape establishes the stress context in which systemic metabolic remodeling, including that induced by the KD intervention, may further perturb leukemic cell survival.

The ketogenic intervention suppresses glycolytic and ribosomal proteins in primary leukemic cells

To determine whether KD alters leukemic responses to chemotherapy, we analyzed longitudinal proteomes using repeated-measures mixed-effects models. One model captured diet-dependent changes in the leukemic proteome during treatment, while a second evaluated whether diet modified the response to chemotherapy (diet × chemotherapy interaction). While ketogenic therapy significantly altered 329 proteins in the leukemic proteome (231 upregulated, 98 downregulated; Fig. 3A), only 15 proteins (< 0.3% of the proteome) were shown to change significantly in response to chemotherapy (Fig. 3B). This suggests that KD leaves the core chemotherapeutic response largely intact while selectively enhancing apoptotic priming (CASP3) and further suppressing ribosomal biogenesis (RPL36AL, RPL24, RPL23).

A prominent consequence of ketogenic intervention was the suppression of glycolytic proteins in leukemic cells (Fig. 3A). Key rate-limiting enzymes, including phosphofructokinase (PFKP) and aldolase C (ALDOC), were markedly reduced, constraining the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate and limiting entry into downstream bioenergetic and biosynthetic pathways. Consistent with this, Fig. 3C presents an upstream regulator analysis identifying D-glucose as a significantly inhibited upstream regulator of the KD-associated proteomic signature. This inference is derived computationally from the directionality of proteomic fold changes relative to a curated knowledge base, thereby predicting a state of systemic glucose restriction.

To determine whether ketone bodies could compensate for reduced glucose utilization, we investigated glucose uptake in the monocytic-like AML cell line THP-1, selected for its proteomic similarity to our primary leukemic cells (**Fig. S4**). THP-1 cells cultured in human plasma-like medium (HPLM), with or without supplementation of the ketone BHB, depleted all available glucose within 48 hours, irrespective of BHB presence at 0, 1 or 5 mM (Fig. 3D–E). These findings indicate that physiologically relevant concentrations of circulating BHB neither modulate nor substitute for glucose uptake in monocytic-like AML.

Concomitant with reduced glycolysis, KD induced broad suppression of the translational machinery. Multiple components of both the 60S cytoplasmic ribosomal subunits (RPL36AL, RPL24, RPL23, RPL36, RPL38, RPL10, RPL36A) and the 40S cytoplasmic ribosomal subunits (RPS13, RPS2, RPS14, RPS26) were downregulated, and pathway enrichment analysis identified ‘cytoplasmic translation’ as the most, and only, profoundly repressed process (Fig. 3A, 3F, **Fig. S7**), including translation initiation, elongation, and termination (Fig. 3G). Suppression of ribosome biogenesis reflects a canonical response to energetic stress and nutrient limitation, given the high metabolic cost of ribosome production. (38–40) In parallel, proteins regulating phosphoribosyl pyrophosphate production (PRPS1, PRPS2, and PRPSAP2) were reduced (Fig. 3A), indicating suppression of nucleotide biosynthesis. As rate-limiting enzymes in nucleotide synthesis, downregulation of PRPS enzymes is expected to restrict DNA and RNA synthesis, further limiting ribosome assembly and anabolic capacity. (40)

Collectively, these findings suggest that short-term ketogenic dietary intervention exerts substantial metabolic pressure on primary leukemic cells. Reduced systemic glucose availability is associated with suppression of glycolytic enzymes, ribosomal proteins, and nucleotide biosynthesis pathways, consistent with energy shortage and reduced anabolic capacity. Given the importance of ribosome assembly in leukemia progression,(15, 16, 39) these findings suggest KD increases metabolic pressure during chemotherapy in AML cells.

Leukemic cells adapt to ketogenic stress through lipid-supported mitochondrial pathways

The suppression of anabolism in KD-treated leukemic blasts indicates a state of energy shortage, prompting us to examine whether cells compensate through alternative pathways. Pathway enrichment analysis revealed that ‘lipid metabolism’ and ‘mitochondrial translation’ were among the most strongly upregulated processes in KD-treated leukemic cells relative to controls (Fig. 3H–I, **Fig. S7**), consistent with switch to lipid oxidation.

Although AML blasts primarily rely on glycolysis for ATP production, they retain metabolic plasticity and can engage mitochondrial FAO under nutrient-limiting conditions. (4) Proteomic profiling revealed broad activation of lipid catabolic pathways in KD-treated blasts (Fig. 3H–I, **Fig. S7**). Lysosomal hydrolases involved in complex lipid and sphingolipid degradation (including GBA1, HEXA, HEXB, GLB1, ARSA, ASAH1, and PPT1) were significantly increased, consistent with enhanced liberation of fatty-acyl

substrates for downstream oxidation, and/or changes in cell membrane remodeling (Fig. 3A). (13) This was accompanied by increased levels of lipid transport proteins such as NPC2 and SCP2, suggesting augmented trafficking of cholesterol and FAs toward mitochondria. Expansion of the lysosomal compartment was further supported by elevated levels of LAMP1, LAMP2, and SCARB2/LIMP-2 (Fig. 3A, Fig. 3H-I).

Consistent with this shift, mitochondrial translational machinery was broadly upregulated. More than twenty mitochondrial ribosomal proteins (MRPs) were increased ($\log_2$ fold change 0.71–2.10; FDR < 0.05), making mitochondrial translation one of the most enriched pathways in KD-treated blasts (Fig. 3A, 3H, 3I). As mitoribosomes synthesize the 13 core components of the electron transport chain encoded by the mitochondrial genome (41), this pattern is consistent with enhanced mitochondrial investment and a functional shift toward FAO-supported OxPhos.

Importantly, this adaptive response occurred alongside elevated apoptotic signaling. Increased CASP3 suggests that, despite engaging lipid-supported mitochondrial catabolism, KD-treated leukemic cells remain energetically stressed and partially committed to apoptosis (Fig. 3B). Together, these data indicate that systemic carbohydrate restriction suppresses glycolytic-based anabolic metabolism while promoting compensatory lipid-supported mitochondrial pathways. This response highlights both the metabolic plasticity of AML blasts (3, 8, 13) and a potential vulnerability to inhibitors of lipid metabolism or mitochondrial function. (8)

Single-cell transcriptomics reveals hierarchical responses to chemotherapy

AML comprises a heterogeneous spectrum of malignant cell states spanning the myeloid differentiation hierarchy, with distinct populations differing in proliferative capacity, metabolic wiring, and therapy sensitivity. (4, 7, 9, 14, 20, 42) To dissect how leukemic cell states respond to chemotherapy *in vivo*, we performed large-scale single-cell transcriptomic profiling of PBMCs collected before ($n = 14$) and during induction chemotherapy ($n = 15$) (Fig. 4A). Across 2,047,464 high-quality cells and 5,101 targeted transcripts, mapping to the Van Galen single-cell RNA-seq AML atlas (42) resolved six malignant populations: HSC-like (110,708 cells; 5.4%), progenitor-like (200,355 cells; 9.8%), GMP-like (110,899 cells; 5.4%), promonocyte-like (3,984 cells; 0.2%), monocyte-like (319,695 cells; 15.6%), and cDC-like (63,226 cells; 3.1%), alongside ~ 60% non-malignant hematopoietic cells (Fig. 4A, 4B). This dataset represents, to our knowledge, one of the largest single-cell transcriptomic datasets in human AML. (42, 43) Marker expression confirmed expected lineage programs, including stem-associated transcripts in HSC-like cells (CD34, PROM1/CD133, MSI2, ERG) and monocytic differentiation markers in monocyte-like blasts (CD14, TREM1, STAB1, PLAUR) (**Fig. S8**). Dimensionality reduction separated malignant from non-malignant populations while resolving discrete leukemic clusters along the differentiation axis (Fig. 4B, 4C, **Fig. S9**), providing a framework for examining state-specific responses to chemotherapy and metabolic intervention.

To define the molecular context of dietary intervention, we profiled the transcriptomic response of primary AML cells to induction chemotherapy. Prior single-cell studies have shown that AML exhibits heterogeneous, state-dependent responses, including persistence of quiescent stem-like populations. (44–46) In our study across > 800,000 primary leukemic cells, cytarabine–anthracycline treatment induced a conserved transcriptional stress program across all malignant states, characterized by DNA damage signaling and p53-dependent apoptotic pathways (*DDB2*, *MDM2*, *PHLDA3*, *BAX*, *BBC3/PUMA*), together with stress effectors such as *CDKN1A* and *GADD45A* (Fig. 4D, E, F, G, H, I). This canonical transcriptional response mirrors our proteomics findings (Fig. 2), supporting coordinated activation of genotoxic and stress-adaptive programs.

Despite this shared response, transcriptional programs diverged by differentiation state. Immature HSC-like and progenitor-like populations (Fig. 4D, 4E) adopted a constrained, slow-cycling phenotype, characterized by downregulation of replication and cell-cycle regulators (*MCM3*, *MCM5*, *TMP0*, *CEBPA*) while upregulating DNA repair genes (*XPC*, *POLH*, *DDB2*), apoptotic priming factors (*BAX*, *BBC3/PUMA*), and redox-buffering enzymes (*SLC7A11*, *CBS*, *CBSL*). These cells also upregulated developmental regulators associated with stemness programs, including *GLI2*, *PBX1*, *ISL1*, and *LIN28A* (Fig. 4D, 4E), consistent with therapy-persistent phenotypes. (45, 46) Notably, *GLI2* was the most upregulated transcript by fold change in both HSC-like (logFC = 3.86, adj. $p < 0.0001$) and progenitor-like cells (logFC = 3.45, adj. $p < 0.0001$), consistent with activation of Hedgehog signaling. This pathway has been implicated in LSC maintenance and chemoresistance,(47) and its pharmacologic inhibition has demonstrated clinical benefit in AML.(48)

In contrast, more differentiated leukemic populations, including GMP-like (Fig. 4F), promonocyte-like (Fig. 4G), monocyte-like (Fig. 4H), and cDC-like (Fig. 4I) cells, exhibited broader transcriptional remodeling in response to chemotherapy. While retaining core DNA damage elements (*DDB2*, *MDM2*, *PHLDA3*), these populations preferentially activated inflammatory and cytokine networks (*CXCL5*, *CXCL9*, *CCL17*, *IL6*, *IL10*, *IL17A*), interferon signaling (*IFNA1*, *IFNB1*, *IFNL1*), immunomodulatory genes (*CD80*, *PDCD1LG2*), extracellular matrix and tissue remodeling factors (*MMPs*, *COL4A1*, *POSTN*), developmental ligands (*WNTs*, *GLI2/3*), and receptor tyrosine kinases (*EGFR*, *PDGFRA*, *VEGFC*). The expanded transcriptional response was most pronounced in GMP-like and promonocyte-like populations (Fig. 4F, 4G) likely reflecting their high proliferative activity and consequent susceptibility to genotoxic stress imposed by cytarabine–anthracycline therapy.

Therefore, chemotherapy response in AML is hierarchically organized: primitive compartments primarily converge on replication-arrested, stress-adapted states associated with persistence, whereas differentiated populations engage broader inflammatory and signaling programs.

Diet-induced metabolic stress selectively remodels stem and progenitor leukemic compartments

To assess how systemic metabolic intervention influenced leukemic cell states, we profiled longitudinal transcriptomes using repeated-measures mixed-effects models, capturing diet-dependent effects (Fig. 4D-I) and diet x chemotherapy interactions (**Fig. S10**) across malignant populations. While chemotherapy induced transcriptional responses across all populations (predominantly upregulation, Fig. 4D-I, left panels), ketogenic intervention elicited a strikingly state-dependent response (Fig. 4D-I, right panels). The dietary pressure caused extensive transcriptional repression in immature compartments, including HSC-like (715 transcripts adj-p < 0.05, 14%) (Fig. 4D), progenitor-like (1043 transcripts adj-p < 0.05, 20%) (Fig. 4E), and GMP-like (1434 transcripts adj-p < 0.05, 28%) (Fig. 4F) states. The dominant pattern was widespread downregulation, indicating that diet-induced metabolic stress selectively constrains stem-like leukemic compartments. In stark contrast, more differentiated leukemic populations showed virtually no differential expression in response to KD (0 transcripts in pro-monocyte-like, 54 transcripts in monocyte-like, and 0 transcripts in cDC-like cells) (Fig. 4G-I).

HSC-like cells exhibited a pronounced ketogenic response. In contrast to the adaptive lipid-mitochondrial remodeling observed in bulk proteomics (Fig. 3), these cells predominantly downregulated transcripts without evidence of compensatory FAO or OxPhos activation, consistent with metabolic inflexibility. Repression spanned carbohydrate (Fig. 4J), protein (Fig. 4K), and fatty acid metabolism (Fig. 4L), in line with previously described metabolic inflexibility in LSCs, (4, 23) here observed *in vivo*. Core lipid-handling genes, including *PPARG*, *FABP* family members (*FABP2*, *FABP3*, and *FABP4*), and *LPL*, were uniformly suppressed (Fig. 4D, Fig. 5). In parallel, glucose uptake and utilization were curtailed at multiple nodes, with downregulation of *SLC2A1* (GLUT1) and *SLC5A2* (SGLT2), alongside repression of glycolytic enzymes (including *LDHAL6A*) and the pentose phosphate pathway (*TKTL1*). Notably, *SLC2A1* downregulation in leukemic cells has also been observed in mice exposed to ketogenic diets.(49) Growth factors (*IGF-1*, *IGF-2*) and nutrient-sensing (*GHR*, *IRS4*, *LEPR*) pathways linked to systemic anabolic signaling were also reduced. Amino acid metabolism also showed a selective suppression. Glutamate sensing receptors (*GRIK3*, *GRIK4*, *GRM5*) and high affinity transporters (*SLC1A1*, *SLC1A2*, *SLC1A3*) were downregulated, suggesting reduced glutamate uptake. By contrast, other amino acid transporters (*SLC1A5*, *SLC7A5*, *BCAT1/2*) remained unchanged, potentially preserving core flux of branched-chain and large neutral amino acids to sustain AML metabolism (Fig. 4D). No amino acid-related pathways were upregulated, consistent with an overall reduction rather than compensatory enhancement of amino acid metabolic capacity.

In contrast to more differentiated populations, mitochondrial metabolic adaptation failed to engage in HSC-like leukemic cells. Pathway-level analyses showed no induction of β -oxidation (adj-p > 0.05), indicating a lack of compensatory lipid utilization despite systemic nutrient stress. Consistent with this, acetate assimilation into acetyl-CoA may have been impaired, with downregulation of *ACSS1* and *ACSS3* (Fig. 5). *CPT1A*, the rate-limiting enzyme for mitochondrial FA import, was the notable exception (Fig. 4L, Fig. 5), suggesting residual pressure to maintain lipid flux despite broader suppression of lipid metabolism. Consistent with this, *CPT1A* was significantly increased at both the RNA and protein level in leukemic cells isolated from mice on a KD (49), supporting its role as a conserved adaptive response across murine and human contexts. Notably, *CPT1A* has been linked to FA dependence and therapeutic

persistence in AML, suggesting that residual lipid-import capacity may support survival despite broader metabolic restriction (50–52).

Despite broad metabolic repression, HSC-like leukemic cells maintained a selective survival and stemness program (Fig. 4D, Fig. 5). Core transcriptional regulators associated with leukemic persistence, including *MYC*, *RUNX1*, and *FLT3*, were upregulated, consistent with maintenance of stemness, survival, and therapy-resistant programs linked to poor prognosis. (53–55) *MAPK11*, a stress-activated MAPK pathway component engaged during fasting, (56) was also upregulated, suggesting an additional buffering response to metabolic stress. This phenotype was accompanied by suppression of differentiation-associated programs (e.g., *SOX1/2/8/17*, *LEF1*, *FOXD2*, *WNT*) and immune-associated programs (e.g., *CXCR1*, *IL23R*, *PDCD1*, *CTLA4*, *CD28*) (Fig. 4D, Fig. 5). Therefore, by day 3 of chemotherapy, KD had caused a metabolically constrained yet stemness-preserving state in HSC-like cells, characterized by potentially low metabolic flux together with reinforcement of stemness programs under energetic stress. Importantly, the ketogenic intervention did not alter the core chemotherapeutic response, as diet x chemotherapy interactions were minimal at the transcript levels in HSC-like and progenitor-like populations (**Fig. S10**), indicating that chemotherapy-induced transcriptional programs remained largely preserved.

Differentiation state dictates metabolic responsiveness to ketogenic therapy in AML

In contrast to immature leukemic populations, more differentiated compartments, including promonocyte-like, monocyte-like, and cDC-like cells, were largely transcriptionally unresponsive to KD (Fig. 4G-I, right panels). Promonocyte-like (Fig. 4G) and cDC-like (Fig. 4I) cells showed no detectable diet-associated transcriptional response despite robust remodeling under chemotherapy (**Fig. S10**). This pattern is consistent with canonical hematopoietic differentiation, in which cells transition from glycolysis-dependent stem states toward greater reliance on mitochondrial OxPhos as they mature. (57) Therefore, while HSC-like cells were highly susceptible to ketogenic stress, differentiated myeloid populations appeared functionally adaptable, relying on pre-existing enzymatic capacity to buffer energetic perturbations without requiring broad transcriptional rewiring (Fig. 4G, 4I, Fig. 5).

Monocyte-like leukemic cells (Fig. 4H) exhibited a limited but discernible response, with only 54 differentially expressed transcripts (adj-p < 0.05), indicating selective pathway engagement rather than global reprogramming. Within this restricted response, lipid metabolism emerged as a focal node, with upregulation of *FADS2*, *FADS1*, and *FAAH* (**Fig. S10**), consistent with the lipid-associated proteomic signature observed in bulk analyses (Fig. 3). In contrast, protein metabolic pathways remained suppressed, indicated by changes in multiple transcripts (**Fig. S10**), suggesting prioritization of energy production over biosynthetic activity. This configuration is consistent with a metabolically flexible, OxPhos-reliant state, in which monocyte-like cells adapt through targeted metabolic adjustments rather than widespread transcriptional remodeling.

Taken together, these findings define a model of distinct metabolic responses across leukemic cell states, with differentiated populations exhibiting adaptive flexibility and stem-like compartments remaining constrained and potentially vulnerable to metabolic targeting (Fig. 5).

DISCUSSION

Our study demonstrates that systemic nutrient manipulation can rewire the metabolic landscape of AML during chemotherapy in patients, exposing dependencies not readily captured by pathway-targeted approaches. More broadly, organism-level metabolic state emerges as a functional lever for interrogating cancer cell-state plasticity *in vivo*. In AML, a single dietary perturbation triggered divergent responses across the leukemic hierarchy: differentiated blasts adapted by increasing lipid-supported mitochondrial metabolism, whereas stem-like populations remained metabolically constrained and reliant on residual survival programs. These findings position systemic metabolic intervention not only as a supportive strategy, but as a means to uncover actionable dependencies in the most treatment-resistant compartments of human leukemia.

Carbohydrate-restricted ketogenic intervention combined with anthracycline-cytarabine chemotherapy imposed a coordinated energetic constraint on leukemic cells, reducing circulating glucose, insulin, and gluconeogenic amino acids while suppressing growth and pro-anabolic signaling, including ribosome biogenesis, and protein and nucleotide biosynthesis. This state resembles canonical responses to nutrient limitation,(40, 56, 58) but occurs in the context of chemotherapy-induced apoptotic priming. Metabolically flexible populations adapted by engaging lipid-supported mitochondrial metabolism, including FAO, mitoribosome biogenesis, and OxPhos. Notably, this metabolic program is concordant with that observed in mice fed ketogenic diets, in which leukemic cells similarly shift toward lipid-metabolism.(49) This proteome-driven adaptation highlights the intrinsic plasticity of AML blasts (4, 9) and reinforces prior evidence that mitochondrial and lysosomal pathways represent actionable vulnerabilities (3, 4, 6, 8, 13, 49) that may be exploited in combination with systemic metabolic interventions.

In contrast, HSC- and progenitor-like leukemic cells, which likely include therapy-resistant LSCs,(42) failed to activate compensatory mitochondrial programs. Instead, they exhibited widespread transcriptional repression across carbohydrate, protein, and lipid metabolism, consistent with broad energetic limitation and impaired adaptive fuel switching. Despite this, these cells preserved core stemness regulators and stress-buffering pathways while silencing differentiation and immune programs. This defines a paradoxical state of halt and persistence: constrained yet poised for self-renewal. Targeting residual survival pathways, through BCL-2 inhibition (venetoclax), mitochondrial-targeted approaches affecting complex I blockade (metformin), FAO inhibitors (etomoxir), or FMS-like tyrosine kinase 3 (FLT3) inhibitors may selectively destabilize this compartment and induce collapse.(14, 21, 49)

Our findings define key principles of metabolic vulnerability in AML. First, ketogenic intervention acts as a multi-faceted metabolic bottleneck, simultaneously constraining glycolysis, amino acid availability, and translational capacity. By imposing systemic nutrient limitations, it amplifies intrinsic metabolic dependencies of proliferative AML blasts. Glycolysis and ribosome biogenesis are central to leukemic proliferation, supporting nucleotide synthesis, protein translation, and biomass accumulation (12). Ribosomal assembly is likewise essential: its disruption impairs leukemia progression *in vivo* and sensitizes AML cells to therapy (16, 18, 19). Consistently, KD suppressed ribosomal programs and constrained nucleotide biosynthesis in patients, recapitulating these vulnerabilities *in vivo*. Given that ribosome production can consume up to 80% of cellular energy,(40) this suppression is expected to markedly limit anabolic growth, underscoring the dependence of highly proliferative AML cells on coupled metabolic and translational programs. To our knowledge, this is the first demonstration of these effects in AML patients undergoing a metabolic intervention.

Second, our data refine a central problem in leukemic stem biology: how therapy-persistent cells sustain energy under stress. Therapy-resistant AML cells rely on amino acid– and fatty acid–fueled mitochondrial OxPhos to survive treatment (20), which underpins the use of BCL-2 inhibitors such as venetoclax to disrupt this dependency (14, 21, 22). In particular, glutamine is a key substrate supporting OxPhos via the glutamine–glutamate axis in LSCs (5). In this context, ketogenic intervention altered glutamate handling in HSC-like leukemic cells at the transcript level, with downregulation of glutamate receptors and transporters suggesting reduced uptake. These cells did not compensate through alternative amino acid transport pathways, creating a state in which oxidative demand is preserved but substrate availability is constrained, potentially limiting LSC fitness. This gives rise to a therapeutic paradox. On one hand, KD may restrict access to critical metabolic substrates in HSC-like compartments, exposing potentially targetable non-redundant dependencies. On the other, the shift toward FAO and OxPhos in differentiated leukemic blasts under KD mirrors the metabolic phenotype associated with venetoclax resistance in AML (14, 21), potentially promoting a drug-resistant state in these cells. Together, these findings underscore the need to evaluate KD in combination with OxPhos inhibitors or FLT3 inhibitors rather than as a monotherapy adjunct.

Importantly, only three days of induction chemotherapy were sufficient to activate survival and stemness programs in HSC-like cells, independent of dietary intervention. Hedgehog signaling, exemplified by *GLI2* induction, was prominent in immature compartments, consistent with prior links to LSC persistence (48). These findings highlight a dual dynamic: chemotherapy induces resistance programs, while metabolic stress creates potential vulnerabilities. Notably, KD was associated with an energetically constrained yet stemness-reinforced state in HSC-like and progenitor-like cells. Despite broad metabolic shutdown, immature leukemic cells, which likely included LSCs, selectively upregulated *CPT1A*, a step-limiting regulator of mitochondrial fatty acid import and a marker of poor prognosis (50–52). Consistent with this, *CPT1A* upregulation was also observed in leukemic cells isolated from mice fed a ketogenic diet (49). Together, these data suggest that *CPT1A* represents a residual survival dependency that may be targeted, particularly in combination with dietary intervention or BCL-2 inhibition.

Strengths and limitations

To our knowledge, this is the first metabolic intervention trial conducted in patients with acute myeloid leukemia (AML). A key strength is the successful implementation of a defined dietary intervention in real-world hospital settings across two independent centers, demonstrating clear clinical translatability. Importantly, the ketogenic diet was prepared and delivered through standard hospital catering services, showing it can be implemented without specialized infrastructure and therefore has strong potential for scalability across routine oncology care.

The study integrates a randomized clinical design with longitudinal multi-omics profiling, enabling direct linkage between systemic metabolic changes and leukemic cell responses in vivo. This is complemented by single-cell transcriptomics, which resolves cell-state-specific effects that are obscured in bulk analyses, particularly within immature and therapy-resistant leukemic compartments. In addition, the depth and scale of the dataset, among the largest generated in AML to date, allow intra-patient, longitudinal comparisons across distinct leukemic populations. This enables a high-resolution mapping of how whole-body metabolic perturbations translate into cell-intrinsic transcriptional and proteomic responses in human disease.

Several limitations should be considered. The sample size was modest ($n = 20$), which precluded assessment of the primary clinical endpoint (infection rates). The intervention was limited to the induction phase, limiting assessment of longer-term clinical outcomes during consolidation cycles. Treatment heterogeneity and analysis confined to peripheral blood rather than bone marrow may also have influenced the observed responses. In addition, functional validation of leukemic cell death and longer-term adaptation was limited by the early sampling window. Nonetheless, the study provides a clinically grounded framework for interrogating metabolic dependencies in AML and for designing future trials to test prolonged or combination metabolic interventions.

In conclusion, systemic metabolic intervention reveals latent, state-specific dependencies in human AML and provides a framework for coupling nutrient modulation with targeted inhibition of mitochondrial and lipid metabolism to selectively destabilize therapy-persistent leukemic compartments.

METHODS

Ethics approval

This study was approved by the Nepean Blue Mountains Local Health District Human Research Ethics Committee (2020/ETH00061), prospectively registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12620000850976), and was assigned a World Health Organization Universal Trial Number (U1111-1251-0734). All participants provided written informed consent before enrolment, with family involvement where appropriate. The trial was conducted by an academic research team from The University of Sydney. All personnel were certified in International Council for Harmonisation Good Clinical Practice (ICH-GCP), and the study was conducted in accordance with the Declaration of Helsinki

and ICH–GCP guidelines. Participants could withdraw at any time without affecting their clinical care. The Participant Information and Consent Form outlined study objectives, procedures, risks and responsibilities; signed forms were retained by the investigators, with copies provided to participants.

Clinical trial design

We conducted a randomized, controlled, open-label, academic, 2-center trial to assess a short-term ketogenic intervention during induction chemotherapy in patients with newly diagnosed AML. The study was undertaken at two tertiary teaching hospitals affiliated with the University of Sydney (Nepean Hospital and Westmead Hospital, NSW, Australia) within standard inpatient care. Participants were randomized to a ketogenic diet or standard hospital diet during induction therapy. As per study protocol, the primary endpoint was the incidence of major infections; however, the trial did not achieve the prespecified sample size required to adequately evaluate this outcome due to low enrolment. Secondary endpoints presented herein included systemic metabolic parameters (β -hydroxybutyrate, glucose, and insulin concentrations) and integrative multi-omics profiling of patient-derived samples, including transcriptomic, proteomic, and metabolomic analyses.

Randomization

The allocation sequence was generated using a computer-based randomization system (Sealed Envelope, <https://www.sealedenvelope.com/simple-randomiser/v1/lists>). Randomization was performed in permuted blocks with a 2:3 allocation ratio (control:ketogenic) to account for a potentially higher dropout rate in the intervention arm, given the open-label design. Stratification was applied by sex (male or female) and study site (Westmead Hospital and Nepean Hospital). The study coordinator generated the randomization sequence, which was exported as a blinded .csv file and uploaded into REDCap for secure allocation. Investigators remained blinded to the allocation sequence throughout the trial.

Participant recruitment

Participants were enrolled from October 2022 to December 2024. Eligible adults (aged ≥ 18 years) had newly diagnosed AML and were scheduled for standard 7 + 3 induction chemotherapy. All French–American–British (FAB) subtypes were included except M3 (acute promyelocytic leukemia), which is treated with a distinct regimen. Patients with suspected AML were identified by treating hematologists. To obtain diagnostic material without additional procedures, patients were approached before routine bone marrow aspiration and offered pre-consent for collection of surplus marrow for research. Following diagnostic confirmation, eligibility was independently assessed by the research team. Participants received the Participant Information and Consent Form and were able to discuss the study before providing written consent. Declining participation did not affect standard care. Inclusion and exclusion criteria are detailed in **Table S1**, and participant flow is shown in the CONSORT diagram (**Fig. S1**).

Ketogenic diet intervention and implementation

The study protocol specified a carbohydrate-restricted (< 10% energy), moderate-protein, high-fat ketogenic diet (KD), combined with a short fast immediately prior to chemotherapy. The pre-chemotherapy fast was not implemented due to the inability to predict treatment initiation. The ketogenic diet was delivered as specified in the protocol. The ketogenic intervention was adapted from clinical epilepsy protocols and optimized for short-term use during AML induction chemotherapy. It was designed and supervised by a dietitian to ensure nutritional adequacy, palatability and inpatient feasibility. The *ad libitum*, high-fat, low-carbohydrate diet provided $\geq 2,366$ kcal/day, with $\sim 78\text{--}79\%$ fat, $13\text{--}14\%$ protein and $6\text{--}7\%$ carbohydrate (typically < 40 g/day), meeting ketogenic targets ($\sim 80\%$ energy from fat; **Tables S2, S3**). All meals were hospital-prepared using a 1-week rotating menu (three meals and snacks daily), comprising eggs, meat, fish, dairy, added fats (e.g. butter, mayonnaise), non-starchy vegetables and limited fruit. Additional ketogenic snacks (e.g. cheese, nuts, coconut products, ketogenic formulations, protein supplements) supported adherence and intake. Meals were individualized, with flexibility to include externally sourced foods that complied with the diet. Where intake was inadequate, oral ketogenic supplements (KetoCal, Nutricia) were provided. The diet commenced after admission, consent and randomization, prior to chemotherapy to establish ketosis. Participants typically received 2–3 days of the diet before chemotherapy and continued throughout the 7-day induction cycle (mean duration 8.7 ± 2.3 days; **Table S4**), entirely during inpatient care. After completion of the intervention, a standard diet was resumed with gradual carbohydrate reintroduction. Adherence was monitored via food diaries, questionnaires, medical records and serial blood ketone measurements, with regular in-person support. Controls received standard hospital meals per NSW Nutrition Standards for Adult Inpatients (2011), with no carbohydrate restriction and *ad libitum* intake. Most participants completed the protocol, although a minority discontinued early because of palatability (**Fig. S1**).

Chemotherapy and clinical monitoring

Induction chemotherapy (7 + 3) was administered in-hospital over 7 days, with a total admission duration of 28–35 days for side-effect management. The regimen included idarubicin (Zavedos, 12 mg/m^2 intravenously on days 1–3) and cytarabine (Pfizer, 100 mg/m^2 by continuous intravenous infusion on days 1–7) per standard protocols. No other modifications were made except the ketogenic diet. Standard clinical monitoring and sample collection were performed by hospital staff. The research team reviewed medical records daily to track clinical progress, dietary issues, side effects and adverse events, which were graded according to CTCAE v5. Daily records continued until discharge, typically at neutrophil recovery ($\geq 0.5 \times 10^9/\text{L}$, days 28–35). Records were compared between groups to assess differences in symptoms and adverse events during the ketogenic intervention, chemotherapy and recovery.

Blood collection and processing

Bone marrow biopsies were obtained at AML diagnosis and post-induction by trained hematology registrars. For research, 2–20 mL of marrow was collected in K2-EDTA tubes (BD Vacutainer, Cat. no. 367525). Blood was collected during routine clinical care, with an extra tube collected for research purposes. Timing varied across participants and staff, including both fasting and non-fasting samples. Peripheral blood (5–30 mL) was collected via peripheral or central lines into K2-EDTA and SST II tubes (BD Vacutainer, Cat. no. 367958) at baseline, during the ketogenic intervention, throughout chemotherapy, and post-chemotherapy. Plasma and serum were isolated by centrifugation (K2-EDTA: 1,500 g for 15 min at 4°C; SST II: clot $\geq$ 30 min, followed by centrifugation at 2,500 g for 10 min at 4°C) and stored at – 80°C. Mononuclear cells were isolated using SepMate™ PBMC tubes (STEMCELL Technologies, Cat. no. 85450) via density gradient centrifugation (1,200 g, 10 min), washed, counted (Invitrogen Countess 3 automated cell counter, Cat. no. AMQAX2000), resuspended in FBS with 10% DMSO, and frozen before transfer to long-term liquid nitrogen storage. Cell viability exceeded 95% before freezing and upon thawing.

Glucose, ketone, and insulin measurements

Peripheral blood glucose and ketones were measured using the Abbott FreeStyle Optium Neo system (Abbott Diagnostics, Cat. No. 682481) according to the manufacturer's instructions. Serum insulin was quantified with a human insulin ELISA (Thermo Fisher Scientific, Cat. No. KAQ1251) using 50 μ L of serum assayed in duplicate, with absorbance read at 450 nm.

Mass spectrometry of circulating amino acids

Plasma amino acids were measured by LCMS. Briefly, 10 μ L plasma was protein precipitated in extraction buffer at the ratio 3:97 v/v. The extraction buffer consisted of 4:4:1 (v/v) acetonitrile:methanol:water and internal standard phenylalanine-d6 at 2 μ M. Extracted samples were centrifuged for 10 minutes at 4°C 21,000 g. 50 μ L supernatant was transferred into HPLC vials and promptly assayed. LCMS was carried out using a Poroshell 120 HILIC-Z column (2.7 μ m, 2.1x150 mm, Agilent) at 30°C on a Shimadzu LC40 and SCIEX QTRAP6500 + LC-MS/MS system. The pair of mobile phase buffers used were 95:5 (v/v) water:acetonitrile containing 20 mM ammonium hydroxide and ammonium acetate (Buffer A) and 100% acetonitrile (Buffer B) flowed at 200 μ L/min. MS acquisition was performed in positive mode to measure amino acids. The injection volume was 5 μ L. Heated ESI source settings were 5500V and 350°C. Amino acid concentrations were quantified using calibration curve of amino acids standards spiked into 5% BSA as surrogate plasma matrix. Instrument data was extracted using ProteoWizard MSConvert (3.0.21128-7376ae988) and in-house MATLAB (2024a) scripts. Absolute amino acid levels were compared between timepoints (paired longitudinal) and between groups (unpaired longitudinal) using mixed models corrected for multiple testing with FDR < 0.05.

Isolation of leukemic cells

CD33⁺ AML cells were isolated from cryopreserved PBMCs using the EasySep™ Human PE Positive Selection Kit II (STEMCELL Technologies, Cat. #17694). Viable PBMCs (~ 10 million) were thawed, FcR-blocked, and labelled with PE-conjugated anti-CD33 antibody (STEMCELL Technologies, Cat. #60126PE). Cells were then incubated with the PE selection cocktail and RapidSpheres™, and CD33⁺ cells were magnetically separated using the EasySep™ magnet (Cat. #18000). Cell number and viability were assessed by trypan blue using an Invitrogen Countess 3 automated counter (Thermo Fisher Scientific, Cat. no. AMQAX2000).

Flow cytometry

Purity of magnetically isolated CD33⁺ cells was assessed by flow cytometry. Cells (~ 2 × 10⁵) cells were washed in PBS containing 2% FBS and 1 mM EDTA, then incubated 20 min on ice with anti-CD33-PE (STEMCELL, Cat. No. 60126PE) and anti-CD45-V500 (BD Biosciences, Cat. No. 560777), followed by two washes in PBS containing 2% FBS and 1 mM EDTA. Acquisition (~ 10,000 events per sample) was performed on a BD FACsVerse (equipped with 405 nm and 488 nm lasers), with analysis in FlowJo v10.10.0 and FACsVerse software. Bone marrow MNCs (1 × 10⁵) were processed similarly. Cells were gated by side scatter (SSC) versus CD45, and CD45 vs CD33, and marker expression was then analyzed. Cell (%) per gate is presented in Fig. 2A (CD45⁺CD33⁺ cells = 97.5%).

Mass spectrometry data acquisition of leukemic cells proteome

Cellular proteomics was performed on CD33⁺ AML cells collected before and during chemotherapy (three time points). A total of 2 × 10⁶ cells was used per sample, with a minimum of 5 × 10⁵ used when material was limited. Cryopreserved cells were rapidly thawed, washed three times in PBS, and lysed in 30–50 µL 4% sodium deoxycholate, 100 mM Tris-HCl (pH 8), heated at 95°C for 10 min, sonicated 20–30 min, and clarified by centrifugation (20,000 g, 10 min, RT). Protein concentration was measured by BCA assay, and lysates were normalized to 40 µg. Reduction/alkylation was performed with TCEP (10 mM) and CAA (40 mM) at 95°C for 10 min. Trypsin and Lys-C (0.1 µg/µL each) were added and digested overnight (16 h at 37°C, with shaking at 1,000 rpm). Peptides were cleaned using ethyl acetate/TFA extraction and SDB-RPS tip-based solid-phase extraction, washed sequentially, then eluted with 5% of a 25% percent solution ammonium hydroxide/80% acetonitrile and dried in a Genevac evaporator (45°C, 2 h 15 min). Dried peptides were resuspended in 5% formic acid, mixed, centrifuged, quantified by NanoDrop, and normalized to 400 ng/µL. A pooled QC sample was prepared from ≥ 24 representative samples. Peptides were stored at – 30°C until LC-MS/MS analysis. For each sample, ~ 400 ng of peptide in 1 µL was injected onto a Neo Vanquish 4 UHPLC (Thermo Fisher) coupled to an Orbitrap Exploris 480. Peptides were separated on a 50 cm × 75 µm in-house column packed with 1.9 µm C18 ReproSil Pur AQ particles (Dr. Maisch) at 60°C using a 75 min gradient (buffer A: 0.1% formic acid; buffer B: 80% ACN + 0.1% FA) at 300 nL/min: 3–35% B over 66 min, 36–60% B over 2 min, 60–98% B over 2 min, then 98% B

wash for 5 min. Acquisition included one full scan (350–1650 m/z, R = 30,000) followed by 20 DIA MS/MS scans spanning 350–1650 m/z at 30,000 resolution with 25% collision energy.

Mass spectrometry data analysis of leukemic cells proteome

DIA raw files (n = 76) were processed in DIA-NN v2.2.0 with joint analysis, retention time alignment, and match-between-runs. Proteins were identified against the human UniProt reference proteome (UP000005640, downloaded 24 March 2025) plus cRAP contaminants using an in silico spectral library. Trypsin specificity (allowing one missed cleavage), fixed carbamidomethylation, N-terminal methionine excision, and 11 ppm MS1/MS2 tolerance were applied. FDR was 1% at precursor and protein levels. Protein-level quantification was imported into R v4.4.2. Missing values were classified as MAR or MNAR (defined as $\geq 80\%$ between-group asymmetry), retaining proteins with $< 30\%$ missingness or MNAR criteria. Intensities were $\log_2$ -transformed and structured in QFeatures v1.16.0. Imputation used k-nearest neighbors (k = 10) for MAR and QRILC for MNAR. Pooled QC samples were excluded. Differential abundance was analyzed using limma v3.62.2 with empirical Bayes moderation; longitudinal analyses used linear mixed-effects models with participant as a blocking factor. P values were adjusted via Benjamini–Hochberg, FDR < 0.05 significant. Functional interpretation included GO over-representation analysis (clusterProfiler) and canonical pathway/upstream regulator analysis using IPA (QIAGEN, <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>). $\log_2$ fold-changes and adjusted p values informed Core Expression Analysis; pathway directionality used IPA Z-scores. Data visualization employed ggplot2 and ComplexHeatmap.

Cell culture

THP-1 cells were cultured in Human Plasma–Like Medium (HPLM; Gibco, A4899101) with 10% FBS and 1% penicillin–streptomycin, which recapitulate plasma-like metabolic conditions including 5 mM glucose. Ketogenic conditions were generated by adding 1 mM or 5 mM β -hydroxybutyrate (BHB; Merck/Sigma-Aldrich, CAS 625-72-9), with pH adjusted to 7.6 and media sterilized before used. Glucose and ketone levels were verified using an Abbott FreeStyle Optium Neo monitor. Cells were washed, seeded at 2×10^6 per well in 6-well plates (2 mL), in duplicate, and incubated 48 h at 37°C, 5% CO₂. Media glucose and ketones were measured at 24 h and 48 h; cell-free wells served as controls. THP-1 identity was confirmed using Promega GenePrint® 16 (100% concordance with reference profile, Cellosaurus CVCL_0006).

Single-cell transcriptomics (STAMP) analysis of PBMCs

Single-cell transcriptomic profiling of suspension cells was performed using the STAMP protocol adapted for the Xenium Prime In Situ Gene Expression platform (10x Genomics).⁽⁵⁹⁾ STAMP enables

suspension cells, such as PBMCs, to be seeded into defined microwells on Xenium slides using a custom silicone gasket, allowing profiling of > 3 million cells across two slides with a custom metabolic gene panel. Pre- and during-chemotherapy PBMCs were cryopreserved in 90% FBS/10% DMSO.

The cryopreserved samples were thawed at 37°C, washed, counted, and fixed in 10x Genomics fixation buffer (PN-2000517) for 1 h. The fixed cells were then quenched in 1x quenching buffer (10x Genomics; PN-2000516) following the Chromium Fixed RNA Profiling protocol (10x Genomics; CG000478 Rev D, Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling | Official 10x Genomics Support). Xenium slides were coated with 1 mL poly-D-lysine for 1 h at 37°C, washed, and air-dried. Cell number seeded per microwell was calculated to minimise overlapping, and a silicone gasket was applied to facilitate the seeding. The quenched cells were loaded by an experienced user (L.M.) and subjected to a stepwise drying protocol: 4°C 30 min, 25°C 5 min, 42°C up to 1 h, then 42°C 2 h, hold at 22°C. Gaskets were removed, and slides were stored at 4°C with desiccant. Prior to Xenium Prime assays, slides were incubated at 60°C 30 min, equilibrated 7 min, hydrated with 1x PBS, then processed following the 10x Genomics guide (CG000760 Rev C, Xenium Prime In Situ Gene Expression with optional Cell Segmentation Staining User Guide | Official 10x Genomics Support). Custom add-on priming oligos and probes (**Supplementary Material**) were used with Xenium Prime 5K Human Pan Tissue & Pathways Panel. Steps for slide preparation include priming, RNase treatment/polishing, probe hybridization, washes, ligation, rolling-circle amplification, cell segmentation staining, autofluorescence quenching, and nuclei staining. Xenium cell segmentation staining reagents were applied to label nuclei, membranes and cytoplasm for automated morphological segmentation. Slides were scanned and analyzed on the Xenium Analyzer following Xenium Analyzer User Guide (10x Genomics, CG000584 Rev K, Xenium Analyzer User Guide | Official 10x Genomics Support) by an experienced user (D.Z.). Regions of interest encompassing STAMPed areas were selected (Fig. 4), and data acquisition ran 4–5 days depending on region size.

Single-cell transcriptomics (STAMP) data analysis

Quality control was performed on the single-cell transcriptomics data. Low quality cells were filtered based on the criteria of number of unique genes < 10, number of total transcripts < 15, cell area less than 20 or larger than 300. Cell counts were library-size normalized and log-transformed. Dimension reduction including UMAP was performed on the log-normalized expression values. A total of 2,047,464 high-quality cells was retained for analysis. Cell type was annotated through supervised classification using the scClassify method (<https://pmc.ncbi.nlm.nih.gov/articles/PMC7306901/>) with the Van Galen et al. study (42) as the reference data. The Van Galen reference is an acute myeloid leukemia dataset that contains both normal PMBC cells and malignant leukemia cells. This enabled the identification of normal immune cells and six leukemia-associated populations in the single-cell data, including HSC-like (hematopoietic stem cell-like), Prog-like (progenitor-like), GMP-like (granulocyte-monocyte-progenitor-like), ProMono-like (pro-monocytic-like), Mono-like (monocytic-like) and cDC-like (conventional dendritic cell-like). scClassify allows cells to remain unassigned when the confidence, measured by class

probability, is below a certain threshold. The default setting at 0.7 was used. For differential expression analysis, single-cell raw counts were aggregated into pseudobulk by summing within each cell type. The pseudobulk samples were normalized for library size and log2 transformed. Three types of differential expression analysis were performed separately for each leukemia-associated cell type using Limma (<https://pmc.ncbi.nlm.nih.gov/articles/PMC4402510/>). First, the overall effect of chemotherapy was examined across both diet groups. Second, a diet-by-treatment interaction model was fitted and the diet effect at the chemotherapy timepoint was extracted. Third, the same interaction model was used to extract the diet and chemotherapy interaction effect, that is, whether dietary intervention altered the transcriptional response to chemotherapy. For all three models, P values were adjusted using the Benjamini-Hochberg method. Because matched samples from the same patient were present across timepoints, within-patient correlation was estimated and incorporated into the model for all three analyses.

Declarations

Conflicts of Interest.

The authors declare no competing interests.

Author Contributions.

L.F. and S.J.F. conceived the study, designed the trial, and developed the protocol. S.J.F., M.L.C., and G.R. implemented the trial. M.L.C., G.R., T.P., R.H., and K.S. recruited participants, coordinated visits, monitored diet adherence, and collected/processed samples. T.P. and R.H. recorded adverse events. M.L.C., G.R., and A.A.G. performed flow cytometry and insulin ELISAs; M.L.C. conducted cell culture and leukemic cell isolation; M.L.C., G.R., T.P., and R.H. performed metabolic measurements. L.E. performed amino acid measurements and analysis. M.L.C., A.M., and L.S. performed and analyzed cellular proteomics. L.M., K.W., and M.L.C. prepared cell STAMPs; D.Z. and M.L.C. performed transcriptomic staining and Xenium imaging; Y.C., J.Y., and M.L.C. analyzed transcriptomic data. M.V.W. and R.K.B. contributed to clinical and statistical interpretation, respectively; S.J.T. and L.V. served as supervising hematologists at each hospital; L.F. supervised the nutritional intervention and secured funding. M.L.C. led data integration, prepared figures and tables, and, with L.F., wrote the manuscript. All authors reviewed and approved the final version.

Acknowledgements.

We sincerely thank the patients and families for their participation and sample contributions under challenging circumstances. We acknowledge the multidisciplinary clinical and research teams, dietitians, nurses, physicians, pathology staff, and clinical trials personnel at Nepean and Westmead Hospitals,

whose efforts enabled this study. We also thank consumer representatives, including AML survivors and family members, for contributing to study design. We thank 'A Life Plus' and 'Slendier', who donated ketogenic food products (<\$2,000), with no role in study design or analysis, and no authors received commercial benefit. We are grateful to Prof. Tony Cesare for discussions on DNA damage biology. M.L.C. acknowledges NSW Cancer Partners' support for travel to the European Hematology Congress. Funding was provided to L.F. by the NHMRC Investigator Grant (APP1177797) and the Australian Youth and Health Foundation; M.L.C. was supported by Schmidt Futures (Schmidt Science Fellowship) and The University of Sydney (Helen and Robert Ellis Fellowship); S.J.F. was supported by a philanthropic donation from Gary and Marion Krelle and the Nepean Medical Research Fund. In-kind support came from Nepean Blue Mountains Local Health District (dietetic services) and the University of Sydney. We thank the Sydney Informatics Hub and Sydney Mass Spectrometry Facility for experimental/statistical support and training.

Data Availability.

Metabolomic, proteomic, transcriptomic, dietary, and clinical datasets are subject to controlled access in accordance with the study protocol and ethical approvals. De-identified data may be made available to qualified researchers for non-commercial purposes upon reasonable request to the corresponding author, contingent on institutional Research Collaboration Agreement (RCA) approval and relevant ethics approval.

References

1. Jeurkar C et al (2026) Management of Acute Myeloid Leukemia: A Review. *Cancers (Basel)* 18
2. Shokoohi M et al (2025) Targeted and epigenetic therapies for acute myeloid leukemia treatment. *Discov Oncol* 16:1711
3. Addanki S, Kim L, Stevens A (2025) Understanding and Targeting Metabolic Vulnerabilities in Acute Myeloid Leukemia: An Updated Comprehensive Review. *Cancers (Basel)* 17
4. Kreitz J et al (2019) Metabolic Plasticity of Acute Myeloid Leukemia. *Cells* 8
5. Silvestri G (2026) Fueling the Fire: How Glutamine Metabolism Sustains Leukemia Growth and Resistance. *BioMed (Basel)* 6
6. O'Brien C, Jones CL (2025) Unraveling lipid metabolism for acute myeloid leukemia therapy. *Curr Opin Hematol* 32:77–86
7. H. JT et al., Acute myeloid leukemia mitochondria hydrolyze ATP to resist chemotherapy - PubMed. *bioRxiv: the preprint server for biology*, (11/11/(2024)
8. Dembitz V, James SC, Gallipoli P (2025) Targeting lipid metabolism in acute myeloid leukemia: biological insights and therapeutic opportunities. *Leukemia* 39:1814–1823
9. Mesbahi Y, Trahair TN, Lock RB, Connerty P (2022) Exploring the Metabolic Landscape of AML: From Haematopoietic Stem Cells to Myeloblasts and Leukaemic Stem Cells. *Front Oncol* 12:807266

10. I N, KJ H, IS G (2020) R. H, The PI3K-Akt-mTOR Signaling Pathway in Human Acute Myeloid Leukemia (AML) Cells - PubMed. *International journal of molecular sciences* 21, (04/21/
11. Chen WL et al (2014) A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. *Blood* 124:1645–1654
12. Herst PM, Howman RA, Neeson PJ, Berridge MV, Ritchie DS (2011) The level of glycolytic metabolism in acute myeloid leukemia blasts at diagnosis is prognostic for clinical outcome. *J Leukoc Biol* 89:51–55
13. Sokei J, Kanefsky J, Sykes SM (2025) Reprogramming of Fatty Acid Metabolism in Acute Leukemia. *J Cell Physiol* 240:e70000
14. Stevens BM et al (2020) Fatty acid metabolism underlies venetoclax resistance in acute myeloid leukemia stem cells. *Nat Cancer* 1:1176–1187
15. Zhou F et al (2023) A Dynamic rRNA Ribomethylome Drives Stemness in Acute Myeloid Leukemia. *Cancer Discov* 13:332–347
16. Sjövall D et al (2024) Defective ribosome assembly impairs leukemia progression in a murine model of acute myeloid leukemia. *Cell Rep* 43:114864
17. Christensen NV, Knudsen JH, Laustsen C, Bertelsen LB (2025) The effect of 2-Deoxy-D-glucose on glycolytic metabolism in acute myeloblastic leukemic ML-1 cells. *Sci Rep* 15:17685
18. Li W et al (2025) FTO degrader impairs ribosome biogenesis and protein translation in acute myeloid leukemia. *Sci Adv* 11:eadv7648
19. Fooks K et al (2022) EIF4A inhibition targets bioenergetic homeostasis in AML MOLM-14 cells in vitro and in vivo and synergizes with cytarabine and venetoclax. *J Exp Clin Cancer Res* 41:340
20. Farge T et al (2017) Chemotherapy-Resistant Human Acute Myeloid Leukemia Cells Are Not Enriched for Leukemic Stem Cells but Require Oxidative Metabolism. *Cancer Discov* 7:716–735
21. Pollyea DA et al (2018) Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med* 24:1859–1866
22. Lagadinou ED et al (2013) BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell* 12:329–341
23. Jones CL et al (2019) Inhibition of Amino Acid Metabolism Selectively Targets Human Leukemia Stem Cells. *Cancer Cell* 35:333–335
24. Stockard B et al (2021) Cellular Metabolomics Profiles Associated With Drug Chemosensitivity in AML. *Front Oncol* 11:678008
25. Firmanty P, Chomczyk M, Dash S, Konopleva M, Baran N (2024) Feasibility and Safety of Targeting Mitochondria Function and Metabolism in Acute Myeloid Leukemia. *Curr Pharmacol Rep* 10:388–404
26. Cherkaoui S et al (2025) Reprogramming neuroblastoma by diet-enhanced polyamine depletion. *Nature* 646:707–715

27. Yang L et al (2022) Ketogenic diet and chemotherapy combine to disrupt pancreatic cancer metabolism and growth. *Med* 3:119–136e118
28. Ji CC et al (2019) A ketogenic diet attenuates proliferation and stemness of glioma stem-like cells by altering metabolism resulting in increased ROS production. *Int J Oncol*
29. M. RJ et al., Inhibition of Neuroblastoma Tumor Growth by Ketogenic Diet and/or Calorie Restriction in a CD1-Nu Mouse Model - PubMed. *PloS one* 10, (06/08/(2015)
30. Orgel E et al (2021) Caloric and nutrient restriction to augment chemotherapy efficacy for acute lymphoblastic leukemia: the IDEAL trial. *Blood Adv* 5:1853–1861
31. Chen S et al (2026) 16-h fasting optimizes cancer immunotherapy in mice and humans. *Cell Metab*
32. Rajakumar G et al Effect of ketogenic diets on insulin-like growth factor (IGF)-1 in humans: A systematic review and meta-analysis. *Ageing Res Rev* 102, (2024/12/01).
33. Effinger D et al (2023) A ketogenic diet substantially reshapes the human metabolome. *Clin Nutr* 42:1202–1212
34. Drabińska-Fois N, Majcher A, Jagielski P, Borowicz-Skoneczny S, Romaszko J (2026) Alteration in Amino Acid Metabolism After Isocaloric, Energy-Restricted Ketogenic Diet in Women with Overweight and Obesity: Randomized KETO-MINOX Trial. *Nutrients* 18
35. Krupka C et al (2014) CD33 target validation and sustained depletion of AML blasts in long-term cultures by the bispecific T-cell-engaging antibody AMG 330. *Blood* 123:356–365
36. Fukushima T, Ueda T, Uchida M, Nakamura T (1993) Action mechanism of idarubicin (4-demethoxydaunorubicin) as compared with daunorubicin in leukemic cells. *Int J Hematol* 57:121–130
37. Tomic B et al (2022) Cytarabine-induced differentiation of AML cells depends on Chk1 activation and shares the mechanism with inhibitors of DHODH and pyrimidine synthesis. *Sci Rep* 12:11344
38. Maehama T, Nishio M, Otani J, Mak TW, Suzuki A (2023) Nucleolar stress: Molecular mechanisms and related human diseases. *Cancer Sci* 114:2078–2086
39. Turi Z, Lacey M, Mistrik M, Moudry P (2019) Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. *Aging* 11:2512–2540
40. Shore D, Albert B (2022) Ribosome biogenesis and the cellular energy economy. *Curr Biol* 32:R611–R617
41. Lavdovskaia E et al (2024) A roadmap for ribosome assembly in human mitochondria. *Nat Struct Mol Biol* 31:1898–1908
42. van Galen P et al (2019) Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity. *Cell* 176:1265–1281e1224
43. Whittle J, Meyer S, Lacaud G, Murtuza-Baker S, Iqbal M (2026) Single-cell atlas of AML reveals age-related gene regulatory networks in t(8;21) AML. *Elife* 14
44. Wang F et al (2021) Leukemia stemness and co-occurring mutations drive resistance to IDH inhibitors in acute myeloid leukemia. *Nat Commun* 12:2607

45. Lambo S et al (2023) A longitudinal single-cell atlas of treatment response in pediatric AML. *Cancer Cell* 41:2117–2135e2112
46. Naldini MM et al (2023) Longitudinal single-cell profiling of chemotherapy response in acute myeloid leukemia. *Nat Commun* 14:1285
47. Abraham A, Matsui W (2021) Hedgehog Signaling in Myeloid Malignancies. *Cancers (Basel)* 13
48. Sadarangani A et al (2015) GLI2 inhibition abrogates human leukemia stem cell dormancy. *J Transl Med* 13:98
49. Goupille L et al (2026) Targeting systemic and tumor metabolic balances with ketogenic diets enhance efficacy of therapy in FLT3-ITD acute myeloid leukemia. *Cell Rep* 45:117185
50. Mao S et al (2021) Inhibition of CPT1a as a prognostic marker can synergistically enhance the antileukemic activity of ABT199. *J Transl Med* 19:181
51. Shi J et al (2016) High Expression of CPT1A Predicts Adverse Outcomes: A Potential Therapeutic Target for Acute Myeloid Leukemia. *EBioMedicine* 14, 55–64
52. Ricciardi MR et al (2015) Targeting the leukemia cell metabolism by the CPT1a inhibition: functional preclinical effects in leukemias. *Blood* 126:1925–1929
53. Wesely J et al (2020) Acute Myeloid Leukemia iPSCs Reveal a Role for RUNX1 in the Maintenance of Human Leukemia Stem Cells. *Cell Rep* 31:107688
54. Salvatori B et al (2011) Critical Role of c-Myc in Acute Myeloid Leukemia Involving Direct Regulation of miR-26a and Histone Methyltransferase EZH2. *Genes Cancer* 2:585–592
55. Nogami A et al (2015) FLT3-ITD confers resistance to the PI3K/Akt pathway inhibitors by protecting the mTOR/4EBP1/Mcl-1 pathway through STAT5 activation in acute myeloid leukemia. *Oncotarget* 6:9189–9205
56. Buono R, Longo VD (2018) Starvation, Stress Resistance, and Cancer. *Trends Endocrinol Metab* 29:271–280
57. Mahmoud AI (2023) Metabolic switches during development and regeneration. *Development* 150
58. Cagigas ML et al (2025) The Metabolic Transition Between Fasting and Feeding Alters Aging-Associated Metabolites, Lowers BCAAs, and Stimulates FGF21 Production in Humans. *Aging Cell* 24:e70270
59. Pitino E et al (2025) Single-cell transcriptomics analysis and multimodal profiling through imaging. *Cell* 188:5100–5117e5126

Table 1

Table 1 is available in the Supplementary Files section.

Figures

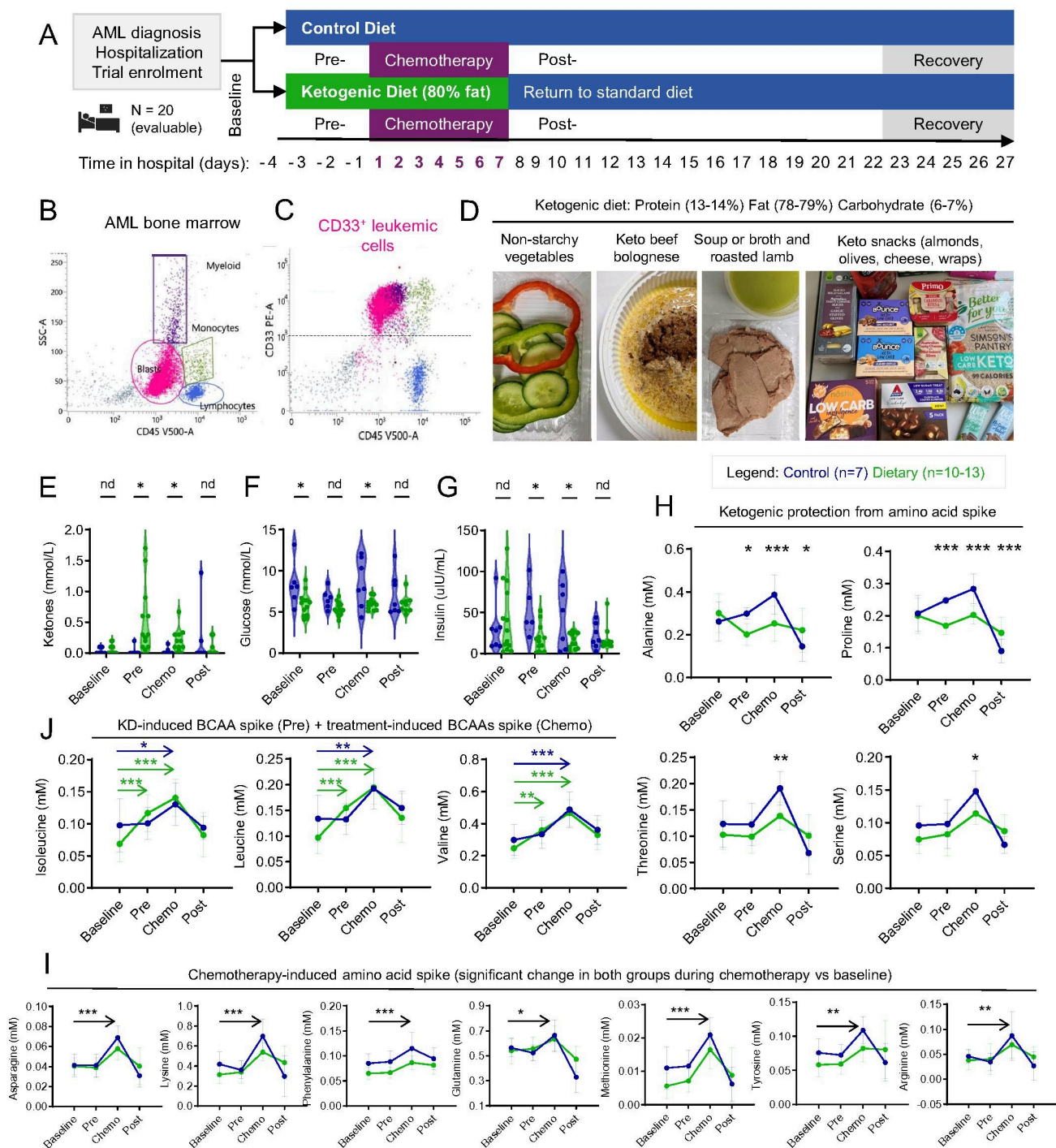


Figure 1

Ketogenic dietary intervention is feasible during induction chemotherapy and remodels systemic metabolism. (A) Study design: 21 participants randomized to control (blue) or ketogenic diet (KD, green) before chemotherapy; samples collected at baseline (Baseline), pre-treatment (Pre), during chemotherapy (Chemo), and post-chemotherapy (Post). (B–C) Bone marrow mononuclear cells from a representative patient by flow cytometry: (B) CD45 vs side scatter, and (C) CD45 vs CD33. CD33⁺

leukemic blasts are shown in pink; 10,000 events/sample. (D) KD intervention images. (E–G) Blood ketone, glucose, and insulin in controls (n=7) and KD (n = 10 completers; n = 13 enrolled) at four timepoints; mixed-model analyses with between-group comparison, FDR (q<0.05; nd = not discovery). (H–I) Blood amino acids measured by mass spectrometry. Mixed models with between-group comparison (H) or within-group vs baseline comparison (I); dot and whiskers = mean ± SD, FDR (*q<0.05, **q<0.01, ***q<0.001, nd = not discovery).

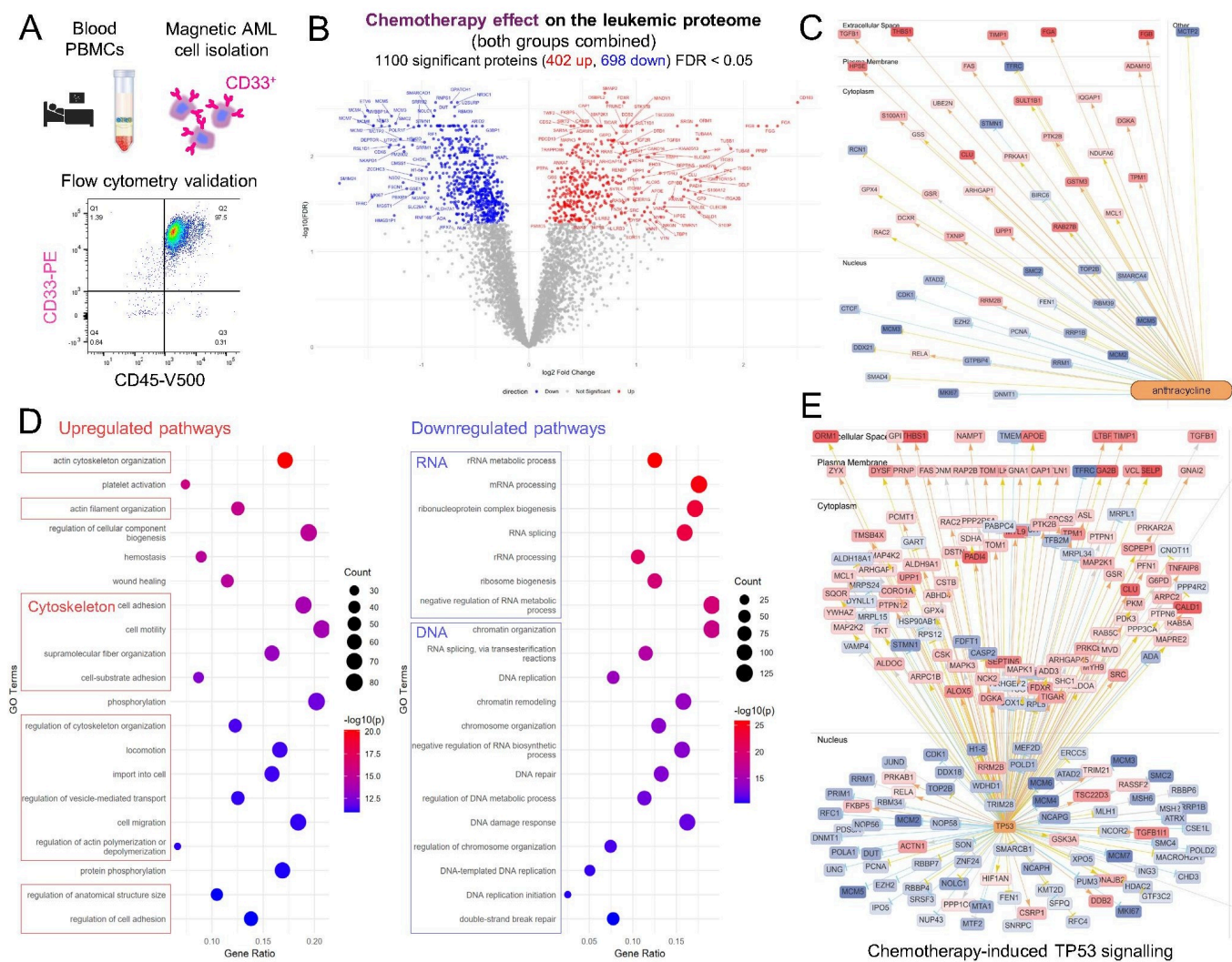


Figure 2

Deep proteomic profiling of primary leukemic cells reveals chemotherapy-induced proteomic remodeling during induction therapy. (A) Leukemic cell isolation: peripheral blood, CD33⁺ magnetic enrichment, purity confirmed by CD45 vs CD33 (10,000 events). (B) Volcano plot of chemotherapy-

induced proteome changes (n=57 samples, three timepoints); up = red, down = blue; longitudinal mixed models with within-group comparison (baseline vs chemotherapy), FDR<0.05. (C) IPA predicts anthracyclines as a significant driver of the proteome phenotype. (D) GO pathway analyses: in the left plot ("Upregulated pathways"), all dots represent pathways enriched among upregulated proteins. In the right plot ("Downregulated pathways"), all dots represent pathways enriched among downregulated proteins. Within each plot, the color gradient shows how significant each term is. More intense colors correspond to larger $-\log_{10}(p)$ values. Circle size indicates protein count and circle color indicate enrichment significance. (E) IPA predicts TP53 as a significant driver of the proteome phenotype.

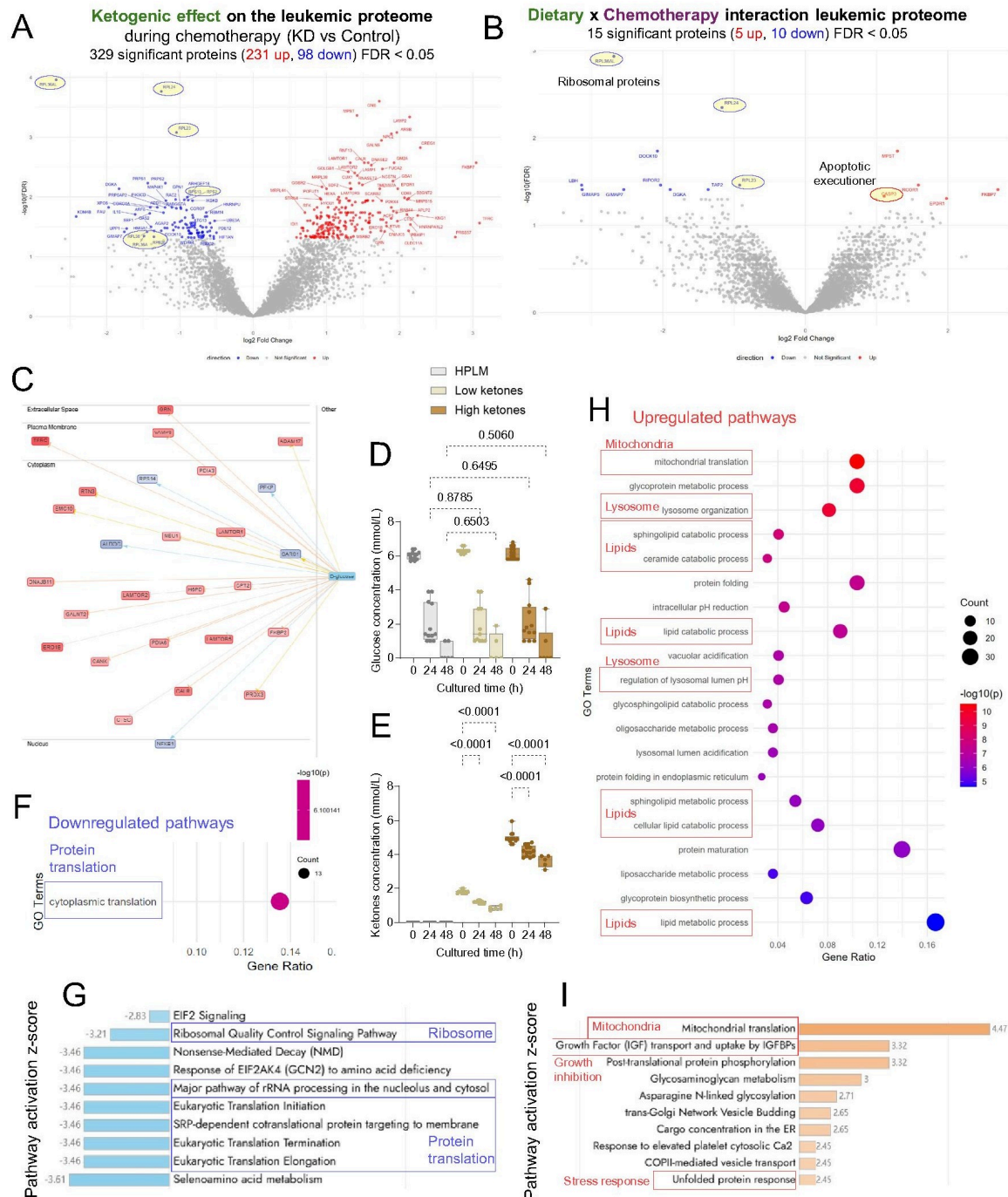


Figure 3

Ketogenic intervention reprograms the leukemic proteome by suppressing translation-associated pathways and promoting lipid metabolism. (A) KD effect on leukemic proteome (KD vs control); red = up, blue = down; mixed-model analyses with between-group comparison, FDR<0.05. (B) Proteins showing a significant diet x chemotherapy interaction in the leukemic proteome; mixed-model analysis with between-group comparison, FDR < 0.05. (C) IPA predicts D-glucose as significant negative driver of KD

proteome. (D–E) Cell culture THP-1 cells: glucose (D) and BHB (E) uptake over 24–48 h in 5 mM glucose (HPLM) $\pm$ 1 or 5 mM BHB; BHB consumed without altered glucose uptake; dots = independent measurements; mixed models with between group (D) and within-group (E), FDR<0.05. (F–I) Pathway enrichment of KD-regulated proteome: F/H = down/up GO terms (circle = protein count, color = p-value), G/I = down/up IPA canonical pathways (FDR<0.05, activation z-score).

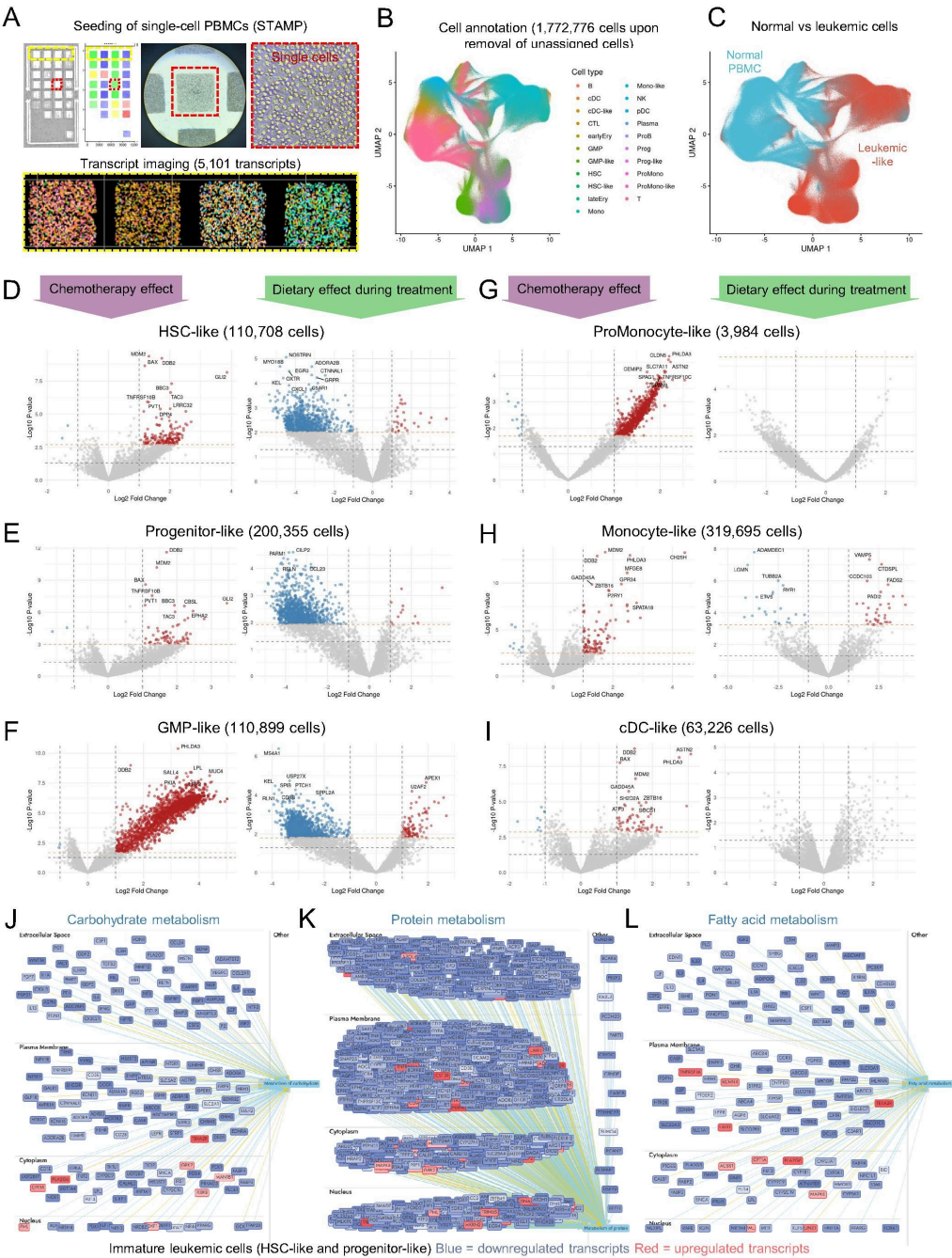


Figure 4

Single-cell transcriptomics reveals hierarchical responses to chemotherapy and dietary intervention.

(A) Single-cell transcriptomics workflow showing seeding of single-cell PBMCs (n=29 PBMC samples from Pre and Chemo timepoints), confirmation of single cells by brightfield microscopy, and transcript imaging (5,101 transcripts). Two identical slides processed in parallel. (B) UMAP mapped to de van Galen scRNA reference; six malignant “like-type” subtypes grouped as leukemic-like. (C) UMAP: leukemic-like (red) vs normal PBMCs (blue). Cell number: 1,772,776 cells upon removal of unassigned cells. (D–I) Volcano plots: mixed model longitudinal within-group (pre vs during chemo, purple) for chemo effects; between-group (KD vs control) for diet effects; up = red, down = blue; FDR<0.05; cell numbers indicated. (J–L) IPA networks indicate altered carbohydrate, protein, and fatty acid metabolism in immature leukemic cells (HSC-like and progenitor-like) under KD; blue = downregulated transcripts, red = upregulated transcripts.

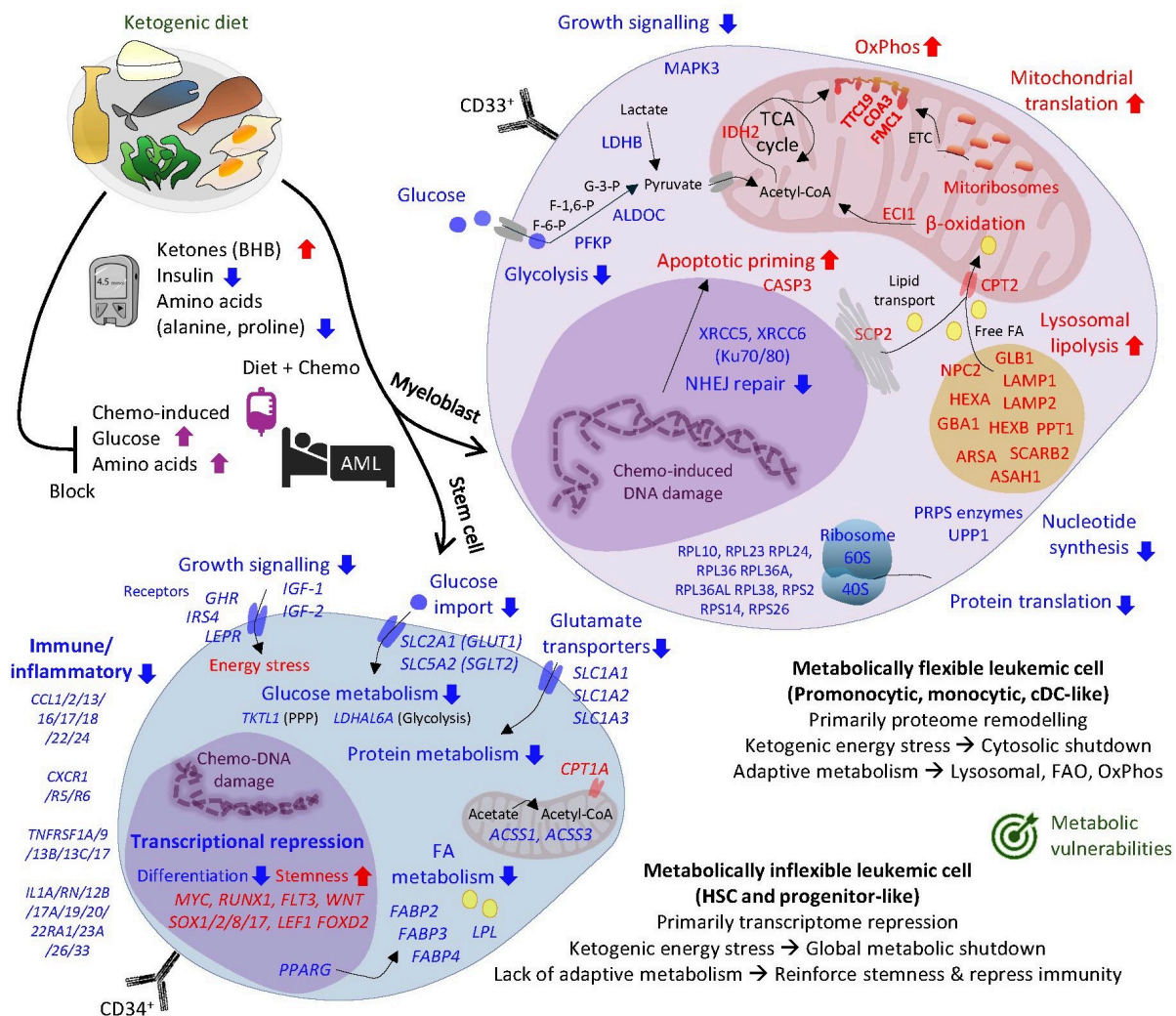


Figure 5

Hierarchical metabolic vulnerabilities in human AML upon dietary intervention. Schematic model integrating systemic metabolic changes induced by a ketogenic diet with cell-state-specific responses in AML during chemotherapy. Ketogenic intervention increases ketones and lowers insulin and selected amino acids while blocking the chemotherapy-associated rise in glucose and amino acids. Metabolically flexible leukemic cells (promonocytic, monocytic, cDC-like) adapt through lysosomal lipolysis, fatty-acid oxidation, mitochondrial translation, and OxPhos, whereas metabolically inflexible leukemic cells (HSC- and progenitor-like) undergo broader repression of glucose, amino acid, fatty-acid, protein, and immune/inflammatory programs. Blue indicates downregulation; red indicates upregulation. *Italic* = transcript effects; normal = protein effects. Key organelles: nucleus, ER, lysosome, mitochondria, ribosomes, mitoribosomes (mitochondrial ribosomes).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.pdf](#)
- [Table1.jpg](#)