key regulator of intestinal permeability via tight junction modulation, was measured by ELISA. A group of 15 healthy individuals were used as a control group. Non-parametric tests with multiple comparisons were used for statistical analysis. **Results:** During the study, 494 blood samples were collected from 136 patients. The highest median zonulin level occurred before conditioning (64 ng/mL), and the lowest at D +30 (55 ng/mL). Compared with controls (21 ng/mL), zonulin was significantly increased as early as pre-conditioning (64 ng/mL, $p < 0.0001$), and remained high at D0 (59 ng/mL, $p < 0.0001$), D+30 (55 ng/mL, $p = 0.0007$), D+60 (60 ng/mL, $p = 0.0002$), and D+90 (61 ng/mL, $p = 0.0002$). Levels were also increased at D+180 (60 ng/mL, $p = 0.0004$) and at GvHD diagnosis (55 ng/mL, $p = 0.0084$). **Discussions and conclusion:** Brazilian patients undergoing allo-HSCT showed persistently elevated zonulin concentrations, when compared with controls, pointing to sustained intestinal mucosal injury throughout the allo-HSCT. This persistence suggests that factors beyond conditioning, such as infections and transplant-related complications, contribute to ongoing barrier disruption. Persistent elevated zonulin may also reflect limited mucosal regeneration during allo-HSCT. Further studies are needed to clarify the mechanisms and clinical impact of this intestinal damage.

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ID - 257

EFFECT OF ERYTHROPOIETIN (EPO) ON GRANULOCYTE-MACROPHAGE COLONY-FORMING UNITS (CFU-GM) IN HUMAN CELL CULTURES

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Introduction: Erythropoietin (EPO) is a hematopoietic cytokine primarily recognized for its role in erythropoiesis. However, recent studies suggest that EPO may also influence other hematopoietic lineages, including granulocytes and macrophages. This study aimed to evaluate the effect of EPO on CFU-GM formation in human bone marrow and peripheral blood samples by comparing two commercial culture media, with and without EPO supplementation. **Aim:** To quantitatively compare CFU-GM formation in cell cultures supplemented with or without EPO, in the context of a medium transition due to discontinuation of the EPO-free formulation. **Material and methods:** Samples were obtained from one healthy donor and four patients undergoing bone marrow transplantation (three with multiple myeloma and one with non-Hodgkin lymphoma). After four days of granulokine stimulation, peripheral blood samples were collected and analyzed by flow cytometry for $CD34^+/CD45^{\wedge}low$ cells using the ISHAGE protocol on a BD FACSCalibur cytometer. Clonogenic assays were performed using MACS^{TM} HSC-CFU methylcellulose media (Miltenyi Biotec), either with or without EPO (3 U/mL). Cultures were incubated for 14 days at 37°C. CFU-GM colonies were counted under an inverted microscope by two blinded evaluators. Statistical analysis was conducted using the paired Wilcoxon test. **Results:** In cultures supplemented with EPO, fresh samples yielded an average of 21.63 CFU-GM colonies, while thawed samples yielded 15.75 colonies ($p = 0.813$). In cultures without EPO, fresh samples yielded 19.47 colonies, and thawed samples yielded 12.67 colonies ($p = 0.813$). No statistically significant difference in CFU-GM formation was observed with the addition of EPO. **Discussion and conclusion:** These findings suggest that EPO does not significantly affect CFU-GM formation in vitro. Therefore, transitioning to a medium containing EPO does not appear to alter the performance of CFU-GM assays in the laboratory setting. The addition of EPO to hematopoietic culture media does not influence CFU-GM formation, supporting the equivalence of both media and validating the continued reliability of CFU-GM testing following the media transition.

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EFFECT OF NATURAL KILLER CELLS CO-EXPRESSING AN IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF AND A CHIMERIC ANTIGEN RECEPTOR TO TARGET NEOPLASTIC B CELLS

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Introduction: Lymphocytic leukemias and non-Hodgkin lymphomas (NHL) associated with B-cell neoplasms shown clinical and biological heterogeneity. Despite therapeutic advances, many cases relapse, highlighting the need for new approaches. T cells modified with Chimeric Antigen Receptors (CAR) targeting CD19, a B-cell surface antigen, have shown remarkable clinical efficacy in immunotherapy against hematologic malignancies. However, CD19-CAR-T cell therapy presents limitations, such as adverse effects including cytokine release syndrome. In this context, Natural Killer (NK) cells emerge as a promising alternative for CAR-based therapy due to reduced toxicity and the feasibility of allogeneic applications. Considering NK cell adoptive transfer, the NK-92 cell line has been evaluated in clinical settings for CAR-based therapy. NK cells express Natural Cytotoxicity Receptors (NCRs), including NKp30, which recognize tumor-associated ligands. The NKp30-dependent cytotoxicity requires association with adaptor proteins bearing ITAM motifs, such as CD3ζ. However, NK-92 cells lack endogenous CD3ζ, impairing signal transduction through NKp30. Thus, engineering NK-92 cells to express CD3ζ and CD19-CAR containing NKp30 as a co-stimulatory domain may enhance their cytotoxic function against malignant B cells. **Aim:** Modification of NK-92 cells

with CD3ζ followed by CD19-CAR containing NKp30 as a co-stimulatory domain, aiming to enhance cytotoxic activity against malignant B cells. **Material and methods:** (i) Construction of lentiviral vectors encoding CD19-NKp30-CAR and CD3ζ, separately; (ii) production of lentiviral particles carrying CD3ζ through transfection of HEK-293T cells; (iii) transduction of NK-92 cells via spinoculation. **Results:** CD19-NKp30-CAR construct was assembled by cloning the domains followed by and ligation using Gibson assembly. The construct was validated by restriction enzyme digestion and PCR amplification of the CAR transgene. This construct will be used to transduce NK-92 cells that were previously modified with CD3ζ via lentiviral transduction. Lentiviral vectors encoding CD3ζ were produced by transfection of HEK-293T cells, associated with helper plasmids, resulting in a titer of 1.335×10^8 TU/mL. Transduction of NK-92 cells reached 17.4% of CD3ζ-positive cells detected by flow cytometry on day three post-transduction. These modified cells were enriched via cell sorting. **Discussions and conclusion:** NK-92 cells can be engineered to the stable expression of CD3ζ, enabling further modification with CD19-NKp30-CAR, thereby enhancing cytotoxic activity against B cells malignancies.

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EFFICACY AND SAFETY OF BELUMOSUDIL VERSUS BEST AVAILABLE THERAPY FOR THE TREATMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE IN THE US POPULATION

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Introduction: To date, no clinical studies have undertaken a head-to-head evaluation of the efficacy and safety of belumosudil vs best available therapy (BAT) for the treatment of relapsed/steroid-refractory chronic graft-versus-host disease (cGVHD) as lines of therapy (LOTs) 3–6. **Aim:** This non-interventional study was designed to estimate the efficacy and safety of belumosudil vs BAT in treating cGVHD patients aged ≥ 12 years who had failed 2–5 prior LOTs via emulation of a target trial. **Material and methods:** Data were collected from patient medical records across 8 sites in the United States (US) between March 1, 2015 and March 27, 2024. Adjusted comparative analysis of cGVHD treatment outcomes following belumosudil vs BAT was performed at the LOT level, with a standardized LOT algorithm applied across all sites. The primary study estimate was defined as the causal ratio of overall response rate (ORR), calculated as the ratio of average overall response (OR) for belumosudil vs BAT at LOTs 3–6 after 6 months of follow-up from the time of LOT initiation. OR was defined as complete (CR) or partial response (PR) of cGVHD based on the 2014 National Institutes of Health consensus criteria, physician-assessed CR or PR, or steroid dose taper of ≥ 50% without disease progression. Death, relapse, and initiation of a new LOT were categorized as failure to respond. The secondary study estimate was the difference in failure-free survival (FFS) between belumosudil and BAT at LOTs 3–6 after 1 year of follow-up. Targeted maximum likelihood estimation, an advanced methodology for causal inference with real-world data, was used to generate all study estimates while adjusting for biases common in non-interventional studies and accounting for correlations among multiple LOTs from the same patient. Adverse events (AEs), including those leading to hospitalization and/or death, observed in were descriptively summarized for belumosudil vs BAT LOTs 3–6 using unadjusted counts and rates per 100 LOT-years. **Results:** A total of 196 patients met all eligibility criteria. Of these, 113 were treated with belumosudil and 83 were not, resulting in 358 LOTs (113 belumosudil, 245 BAT). Baseline characteristics were generally well-balanced across belumosudil and BAT LOTs. The estimated 6-month ORR was 38.7% for belumosudil vs 26.8% for BAT. The 6-month ORR ratio between groups was 1.44 (95% confidence interval [CI]: 1.04, ∞; $p = 0.03$), corresponding to a 44% improvement with belumosudil over BAT. The estimated 1-year FFS was 61.2% for belumosudil vs 47.8% for BAT, resulting in a 1-year FFS difference of 13.5% (95% CI: 1.5%, 100.0%; $p = 0.03$). The AE rate per 100 LOT-years was 41.2% for belumosudil and 51.6% for BAT LOTs 3-6. Belumosudil LOTs had a lower AE rate per 100 LOT-years in 69% of the AE categories. **Discussion and conclusion:** This study demonstrated that belumosudil has superior efficacy compared with BAT in real-world patients with relapsed/steroid-refractory cGVHD. The safety profile of belumosudil-treated patients was consistent with its previously established clinical profile.

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EFFICACY OF CAR-T THERAPY FOR REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: A SYSTEMATIC REVIEW AND METANALYSIS

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