refratário novamente. Para avaliação da resistência, foi solicitado cariótipo para avaliação de evolução clonal e a pesquisa de mutações genéticas de ponto no gene BCR-ABL. Esta foi confirmada pela presença de mutação na porção E459K, localizada no Activation-loop (A-loop) do domínio quinase da proteína ABL, o que dificulta a ligação do Imatinibe ao sítio catalítico e mantém a proteína ativada e em seu estado oncogênico. O mesmo mecanismo provavelmente ocorre para o Nilotinibe. O Ponatinibe, por sua vez, tem demonstrado eficácia contra mutações que afetam o A-loop, sendo assim a nova droga de escolha. **Conclusão:** Dessa forma, devido a possibilidade de resistência ao tratamento de primeira linha e também de segunda linha, temos a necessidade de opções como o Ponatinibe para o tratamento desses pacientes. Cabe ainda ressaltar a necessidade de novas drogas que consigam superar a possível resistência ao tratamento da LMC.

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IMMUNOLOGICAL PROFILES OF CYTOTOXIC CELLS IS ASSOCIATED WITH TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKEMIA

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Patients with Chronic Myeloid Leukemia (CML) who achieve sustained deep molecular response (DMR) to Tyrosine Kinase Inhibitors (TKIs) are eligible for treatment discontinuation, but only 50% of those achieve long-term treatment-free remission (TFR). Current biomarkers that predict resistance to TKIs as well as loss of the molecular response after TKI interruption have not been defined. We hypothesized that antitumoral immunity mediated by Natural Killer (NK) and T cells may contribute to TFR success and result in distinct responses during treatment and after discontinuation. NK and T cells phenotype were correlated with *BCR::ABL1* kinetics and TFR success in a cohort of CML Brazilian patients included in the DES-CML study (Study of treatment discontinuation in CML treated at the Unified Health System), multicenter, prospective, open-label, single-arm, phase 2, non-randomized, ongoing trial, with current data from two Brazilian centers. Using flow cytometry, we evaluated frequency, subtypes, maturation, and receptors expression of NK and T cells from diagnosis to TKI discontinuation. Peripheral blood samples from 20 healthy controls and 68 CML patients were analyzed at different time points: 14 diagnosis (DX), 13 Imatinib failure (F), 7 major molecular response (MMR), 9 DMR, 20 TFR and 5 TFR loss (TFRL). Patients in discontinuation cohort had TKI dose reduced to 50% for 6 months before total suspension. NK cell subtypes were defined as secretory ($CD56^{bright}CD16^{-}$) or cytotoxic ($CD56^{dim}CD16^{+}$). We assessed NK maturation markers (CD57 and NKp80), activation (NKG2D,NKp46,DNAM-1), and inhibition receptors (KIR2DL1,TIGIT,NKG2A). PD-1 checkpoint inhibitor was assessed in CD8 T cells. The frequencies of cytotoxic ($p < 0.05$) and mature ($CD57^{+}$ and NKp80 $p < 0.05$) NK cells as well as NKG2D and DNAM-1 ($p < 0.05$) were lower in the DX, F, and TFRL groups as compared to patients under TKI treatment with DMR, TFR, and healthy individuals. Patients in TFR exhibited a distinct immune profile from TFRL patients that were not influenced by gender, TKI drug, ELTS or SOKAL score. TFR patients presented higher frequency of cytotoxic ($p < 0.05$) and mature ($CD57^{+}$ $p < 0.05$) NK cells, and also NKG2D, DNAM-1,NKp46 ($p < 0.05$) compared with TFRL. Frequency of PD-1 ($p < 0.05$) in T cells was decreased in TFR. At the TKI dose reduction timepoint,TFRL patients already had decreased frequency of cytotoxic ($p < 0.05$) and mature ($CD57^{+}$ $p < 0.05$) NK cells as well as of the activating receptor NKG2D ($p < 0.05$) compared with TFR cases. Conversely, T-cell PD-1 ($p < 0.05$) expression was higher in TFRL than in TFR patients at the dose reduction point. In conclusion, our study indicates that impaired NK cell function and PD-1 T-cell inhibition are major contributors to molecular responses in CML. Patients with sustained TFR exhibit higher frequencies of cytotoxic, mature and activated NK cells, along with lower PD-1 expression in T cells. These profile contrasts with patients who have persistent disease or lose molecular response post-treatment suspension, who demonstrate impaired NK cell function and increased PD-1 expression. Monitoring parameters such as mature NK content and activating receptors, particularly NKG2D, and PD-1 expression during the de-escalation phase of TKI potentially predicts TFR relapse and can be useful for decision-making in TKI interruption.

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GENE AND METABOLITE SIGNATURES IN CHRONIC MYELOID LEUKEMIA PROGRESSION: IMPLICATIONS FOR THERAPEUTIC RESISTANCE AND BIOMARKER DISCOVERY

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of the Philadelphia chromosome (Ph) and/or the *BCR::ABL1* oncogene, which encodes the constitutively activated Bcr-Abl tyrosine kinase protein. Bcr-Abl tyrosine kinase promotes the activation of various cellular signaling pathways, resulting in exacerbated cell proliferation and resistance to apoptosis. CML is initially diagnosed in the chronic phase, characterized by uncontrolled clonal expansion and a high white blood cell count. Without an effective drug intervention, CML may progress to accelerated and blast phases, characterized by high counts of immature myeloid/lymphoid cells in the bone marrow and peripheral blood. The tyrosine kinase inhibitor (TKI) is the gold standard treatment for CML patients. This therapy can induce high rates of hematological, cytogenetic, and molecular remission in CML patients. However, disease progression and therapeutic resistance remain a challenge in improving the prognosis of CML patients. In this context, studies on the cellular and molecular mechanisms involved in TKI resistance and CML progression are still necessary to identify new biomarkers and therapeutic targets. **Aims:** 1) To describe gene signatures that predicts CML progression and response to therapy; 2) To explore the relationship between transcriptomic and the metabolomic plasma profiles from CML patients. **Subjects, Material and methods:** Three publicly available gene expression datasets comparing patients with CML in the chronic phase (CP) and CML patients in the advanced phase (AP) were analyzed. The in silico analysis was performed to identify the set of differentially expressed genes (DEGs) and the gene signature associated with progression. The results were validated through gene expression in patients at different stages of CML using real-time PCR. Additionally, lipid mediators were quantified in plasma samples from 28 CML patients using Liquid Chromatography-Mass Spectrometry (LC/MS). **Results:** The *in silico* analysis by principal component analysis (PCA) revealed the separation of samples from CML patients in CP and AP. The Venn diagram identified the most significant DEGs distinguishing AP from CP in CML patients. Furthermore, DEG analysis highlighted the enrichment of the sphingolipid pathway. Metabolomic analysis of the plasma by LC/MS indicated that CML patients in different phases presented differential profiles of active metabolites. **Conclusion:** Transcriptome analysis revealed gene signatures that have the potential to become biomarkers for CML progression. Additionally, the gene enrichment analysis highlighted the differential bioactive metabolites in CML patients at advanced phases. The analysis of differentially abundant metabolites emerged as a promising criterion to identify patients predisposed to progression and who may not respond effectively to TKIs. **Support:** Supported by CNPq (number 308326/2021-0 and 406361/2021-5); CAPES (process number 001) and FAPESP process number 2021/06841-3 and 2018/00583-0.

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LEUCEMIA MIELOIDE CRÔNICA COM NEGATIVAÇÃO DA MUTAÇÃO T315I APÓS IFN-A: RELATO DE CASO

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Objetivo: Descrever um caso de Leucemia Mieloide Crônica (LMC) com resistência ao Imatinib, apresentando mutação T315I do *BCR-ABL* e o desaparecimento do clone mutado após tratamento com IFN-a. **Materiais e métodos:** Relato de caso por meio de coleta de dados do prontuário do paciente em acompanhamento no Hospital Amaral Carvalho, Jaú (SP). **Relato de caso:** JSS, 69 anos, masculino, diagnosticado com LMC fase crônica em 2009, iniciou tratamento com Imatinib, não atingindo resposta molecular maior (RMM) após 12 meses de tratamento. Perfil mutacional: presença de T315I em 25% das células *BCR-ABL*. O transplante de medula óssea (TMO) alogênico foi contraindicado por ausência de doador. Assim, foi mantido Imatinib, por não haver opções terapêuticas disponíveis na época, atingindo RMM. Mantivemos o medicamento até 2015, quando houve perda de resposta, inclusive hematológica. Iniciamos, então, IFN-a 9.000.000UI 3x/semana, com resposta hematológica. Em 2021, pela indisponibilidade de IFN, iniciamos Hidroxiureia. Em 2024, optamos por nova avaliação do TMO. Realizado novo perfil mutacional, desta vez com ausência de T315I. Há 2 meses iniciou Dasatinib, aguardando a avaliação de resposta. **Discussão:** Apesar do melhor prognóstico dos pacientes com LMC nos últimos 20 anos, com o advento dos inibidores de tirosina-quinase (ITK), equiparando à sobrevida global da população geral, a presença de mutações pontuais no domínio ABL, principalmente a T315I, ainda configura uma necessidade não atendida, uma vez que os ITKs de terceira geração com ação nessas mutações (ponatinib e asciminib) ainda não estão disponíveis, principalmente no contexto público. TMO alogênico ainda é uma das poucas opções para estes pacientes. IFN-a foi amplamente utilizado antes da era ITK, e um dos mecanismos de ação parece aumentar a resposta anti-leucêmica dos Lt citotóxicos, através de seu efeito-imunomodulador, ajudando no reconhecimento de antígenos de superfície das células da LMC (principalmente as stem cells primitivas), como proteinase 3, WT-1, hTERT ou MPO. Existem relatos de casos (poucos) nos quais o IFN-a sozinho, ou em associação com ITKs de