acetilsalicílico (AAS), evoluiu para uso de anagrelida por necessidade de controle citorredutivo. Hemograma realizado no momento da triagem molecular demonstrou uma anemia noromocítica normocrômica (RBC: 3,6 x 10^6/mm^3; Hb: 11,1 g/dL; Ht: 33,5%; VCM: 93,1 fL; HCM: 30,8 pg), leucócitos (WBC: 10.050/mm^3) e plaquetas (356.000/mm^3) dentro do valor de referência. Desidrogenase lática (LDH: 1284,8 U/L), fibrinogênio (546 mg/dL) e ácido úrico (7,7 mg/dL) apresentaram-se elevados. Análise morfológica do esfregaço sanguíneo revelou discreto desvio à esquerda (bastões: 7%; metamielócitos: 5%; mielócitos: 2%), presença de eritroblastos (21/100), linfopenia (21%) e blastos (2%). Apresentou piora do seu quadro e evoluiu a óbito em 2022. A MF pós-PV é um evento complexo que pode ser acelerado por comorbidades e alterações genéticas em especial a combinação da variante driver *JAK2V61F* e o haplótipo 46/1_G em homozigose. Essa homozigose geralmente ocorre como consequência da perda neutra de heterozigosidade do número de cópias do cromossomo 9p, que acompanha as variantes e a reduzem a um estado homozigoto, condição frequente em paciente em evolução neoplásica dessas condições hematológicas. *JAK2V617F* promove a ativação constitutiva de JAK2/STAT, garantido a vantagem clonal. O traço falciforme pode ter exercido papel no estresse eritropoiético e acentuado a remodelação do microambiente medular, favorecendo a deposição de fibrose na medula. Sugere-se um fenótipo neoplásico não hipersplênico devido à ausência de esplenomegalia, entretanto, o aumento de LDH evidencia um turnover celular acentuado. A mudança no tratamento reflete a tentativa de controle dos sintomas, porém a progressão clínica indica refratariedade. **Conclusão:** MF pós-PV é uma condição rara e sua associação com traço falciforme é incomum. A junção desses complexos cenários pode ter contribuído para o curso clínico da doença na paciente. Este caso demonstra a importância do acompanhamento contínuo, bem como o uso de dados genéticos e laboratoriais para orientar as intervenções médicas.

https://doi.org/10.1016/j.htct.2025.104422

ID - 1273

IMMUNE MATURATION AND CHECKPOINT DYNAMICS DURING TYROSINE KINASE INHIBITOR DOSE REDUCTION PREDICT RELAPSE RISK IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA UNDERGOING TREATMENT DISCONTINUATION

LS Binelli [a], LC Palma [b], CAB Garcia [a], M Medeiros [a], AG de Carvalho [a], BL Adjafre [a], LO Marani [a], PS Scheucher [b], JLS Schiavinato [b], PMM Garibaldi [b], RL Pacca [b], FS Seguro [c], RS Tavares [d], LL Almeida [e], RS Welner [f], KBB Pagnano [g], LL de Figueiredo-Pontes [a]

[a] *Fundação Hemocentro de Ribeirão Preto (FUNDHERP), Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*
[b] *Hematology, Hemotherapy and Cell Therapy Division, Department of Medical Imaging, Hematology, and Clinical Oncology, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*
[c] *Division of Hematology and Hemotherapy, Department of Clinical Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil*
[d] *Universidade Federal de Goiás (UFG), Goiânia, Brazil*
[e] *Hospital Amaral Carvalho (HAC), Jaú, Brazil*
[f] *Division of Hematology and Oncology, Department of Medicine, University of Alabama, Birmingham, United States*
[g] *Centro de Hematologia e Hemoterapia (Hemocentro), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil*

Introduction: In Chronic Myeloid Leukemia (CML), patients who achieve sustained deep molecular response (DMR) to Tyrosine Kinase Inhibitors (TKIs) are eligible for treatment discontinuation, but only 50% of these patients maintain long-term treatment-free remission (TFR). Biomarkers that predict TFR loss (TFRL) of the molecular response after TKI interruption have not been defined to date. **Objectives:** We hypothesized that antitumoral immunity mediated by Natural Killer (NK) and T cells may contribute to TFR success. **Material and methods:** 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, a multicenter, prospective, open-label, single-arm, non-randomized, phase 2 clinical trial of TKI discontinuation, conducted in Brazilian public healthcare system (SUS). 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 88 CML patients were analyzed at different time points: 14 diagnosis(DX), 13 Imatinib failure(F), 10 major molecular response(MMR), 20 DMR, 20 TFR and 5 TFR loss (TFRL). Patients in discontinuation had TKI dose reduced to 50% for 6 months before total suspension. **Results:** Patients who successfully maintained TFR exhibited more functional and mature immune phenotype compared to those who relapsed. TFR patients showed higher frequencies of cytotoxic NK cells (CD56dim), mature NK (CD57$^+$), and activating receptors (NKG2D, DNAM-1, NKp46). They also exhibited increased CD8$^+$ effector memory T cells, lower CD8$^+$ naïve T cells, and reduced PD-1 expression on NK and CD8$^+$ T cells, indicating an activated and immunocompetent profile compared to TFRL patients. In contrast, even during full-dose DMR (DMR100), TFRL patients had reduced total NK cells, CD56dim NKs, CD56$^+$CD57$^+$ NK, and CD11b$^+$CD27$^-$ subsets. They also exhibited lower expression of activating receptors (NKG2D, DNAM-1, NKp46). CD8$^+$ T cells from TFRL patients showed decreased central memory, effector memory, and terminal effector subsets, indicating early impairment of the cytotoxic compartment. During the 50% dose reduction phase (DMR50), TFRL patients presented elevated frequencies of immature NK subsets (CD11b$^-$CD27$^+$) and increased expression of inhibitory receptors: TIGIT and PD-1 on NK cells, and PD-1, CTLA-4, TIGIT on CD8$^+$ T cells. This phase also showed increased naïve

CD8+ T cells, reinforcing immune immaturity. Frequencies of mature and cytotoxic NK cells (CD56dim, CD57+, NKp80), as well as NKG2D and DNAM-1, were lower in DX, F, and TFRL groups compared to DMR100, TFR, and healthy controls. PD-1 and CTLA-4 expression on CD8+ T cells was more pronounced in these groups. No significant differences were found in KIR2DL1 and NKG2A on NK cells or CD3, CD4, CD8 expression on T cells. **Discussion and conclusion:** These findings suggest that maturation and activation of cytotoxic compartments are crucial for disease control and TFR success. In contrast, immature and exhausted immune profiles may predispose to relapse. Immune dysregulation precedes molecular relapse, reinforcing the role of longitudinal immune monitoring. Progressive loss of mature NK cells and increased checkpoint expression may define an immunological risk profile for TFR failure. Immune assessment during dose reduction could guide clinical decisions on TKI cessation and support individualized strategies in CML based on immunological profiles.

https://doi.org/10.1016/j.htct.2025.104423

ID - 1690

IMMUNE SENESCENCE AND EXHAUSTION PHENOTYPE MARKED BY LOSS OF NKG2D AND DNAM-1 NK ACTIVATING SIGNALING AND INCREASED TIM-3 T CD8 CHARACTERIZES ADVANCED MYELOFIBROSIS

M Medeiros [a], IC Arruda [a], AG de Carvalho [a], CAB Garcia [a], LS Binelli [a], BL Adjafre [a], PS Scheucher [b], JLdS Schiavinato [b], CL Araujo [a], AS Lima [b], RL Pacca [b], LC Palma [b], LL de Figueiredo Pontes [b]

[a] *Hematology, Hemotherapy and Cell Therapy Division, Fundação Hemocentro de Ribeirão Preto (FUNDHERP), Ribeirão Preto, Brazil*
[b] *Hematology Division, Department of Medical Imaging, Hematology, and Clinical Oncology, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*

Introduction: Myelofibrosis (MF), driven by JAK/STAT signaling mutations in hematopoietic stem cells, has the worst prognosis among myeloproliferative neoplasms (MPNs), progressing from pre-fibrotic MF (PFMF) to overt MF (OMF). Although additional mutations promote clonal expansion, progression cannot be fully explained by clinical- mutational scores. Exposure to the leukemic inflammatory microenvironment may impair immune surveillance by cytotoxic cells such as Natural Killer (NK) and T lymphocytes, fostering disease advancement. **Objectives:** To investigate immune dysfunction across clinical and molecular MF subtypes, focusing on NK and T lymphocyte phenotype, maturation, and receptor expression, and to examine the leukemic microenvironment effects using a JAK2V617F murine model. **Material and methods:** We performed immunophenotyping of NK and T cells (frequency, maturation, and activation/inhibitory receptors) in peripheral blood mononuclear cells from untreated MF patients (13 PFMF, 16 OMF; 13 JAK2 wild-type, 18 JAK2-mutated) admitted at Ribeirao Preto Clinical Hospital (August 2022 – December 2024), and from age-matched healthy donors. To further explore the effects of soluble factors from the leukemic microenvironment, we established a chimeric murine model of JAK2V617F- driven MPN. Healthy recipient mice were transplanted with total bone marrow from JAK2V617F donor mice following sublethal irradiation (n = 4 MPN-exposed, 6 control- exposed). Four weeks post-transplant, spleen cells were collected, and NK and T cells were immunophenotyped. **Results:** MF patients exhibited increased frequencies of NK cells (CD45hiCD3-CD19-CD56+) compared to healthy donors, particularly CD56bright and immature subsets (CD27-CD11b-, CD27+CD11b-), and reduced frequencies of CD56dim, cytotoxic (CD27-CD11b+), and hypermature (CD57+NKp80+) NK cells. There was a decrease of NKG2A (total, cytotoxic, and hypermature NK cells) and NKp46 (total and hypermature NK cells) receptors. T cell maturation was also impaired, with reduced frequencies of stem central memory (CD45RO-CCR7+CD62L+CD27+CD45RA+) and central memory (TCM: CD45RO+CCR7+CD62L+CD27+CD45RA-) subsets in CD4 cells, and TCM in CD8 cells. Terminally differentiated effector memory (CD45RO-CCR7-CD62L-CD45RA+) cells increased in both lineages. CD8 T cells also showed decreased NKG2D expression. During disease progression from PFMF to OMF, there was a further increase in hypermature NK cells and downregulation of DNAM-1 and NKG2A. JAK2-mutated patients showed decreased expression of KIR2DL1 (total and hypermature NK), reduced CD4 T cells, increased CD8 T cells, and decreased CD8 TCM cells. The MPN-exposed group exhibited reduced frequencies of mature NK cells (CD45hiCD19-CD3-CD122+NK1.1+CD49b+NKp46+), NKG2D expression, and T cell frequencies (CD45hiCD19- NK1.1-CD3+), but increased CD8 T cells, with elevated TIM-3 expression.

Discussion and conclusion: These findings reveal distinct immune signatures associated with MF progression and JAK2V617F. NK cell dysfunction, characterized by impaired maturation and loss of activating receptors, may compromise immune surveillance and promote leukemic transformation. Concurrently, T cell alterations suggest chronic inflammation and immunesenescence, particularly in JAK2-mutated cases. The murine model supports a direct role for JAK2V617F-mutant hematopoiesis in driving immune exhaustion. Collectively, our data highlights the contribution of immune dysfunction to MF pathogenesis and progression, providing insights into how molecular heterogeneity may influence disease.

https://doi.org/10.1016/j.htct.2025.104424

ID - 3091

IMPACT OF ABCB1 AND ABCG2 POLYMORPHISMS ON TROUGH IMATINIB CONCENTRATION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

FCA de Moraes [a], VKT Sano [b], JB Gazabón [c], M Kreuz [d], LR Fernandes [e], FAK Kelly [f], RMR Burbano [g]