UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

# INVESTIGAÇÃO DE VARIANTES EM GENES DE FARMACOGENÉTICA EM ASSOCIAÇÃO COM A RESPOSTA AO TRATAMENTO COM IMATINIBE DE PACIENTES COM LEUCEMIA MIELOIDE CRÔNICA EM UMA POPULAÇÃO MISCIGENADA DO BRASIL

KARLA BEATRIZ CARDIAS CEREJA PANTOJA

BELÉM
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KARLA BEATRIZ CARDIAS CEREJA PANTOJA

Tese de doutorado submetida ao Programa de Pós-Graduação em Genética e Biologia Molecular da UFPA como requisito para obtenção do grau de Doutor em Genética e Biologia Molecular

Orientador: Prof. Dr. Ney Pereira Carneiro dos Santos.

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Tese apresentada ao Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Pará, como requisito para a obtenção do título de Doutor.

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(Louis Pasteur)

**RESUMO**

A Leucemia Mieloide Crônica (LMC) é a proliferação anormal da linhagem granulocítica, ocasionada pela translocação recíproca e equilibrada entre os braços longos dos cromossomos 9 e 22 nas regiões q34 e q11 e a formação do cromossomo Philadelphia (Ph). O tratamento de primeira linha da LMC é realizado com o inibidor de tirosina-quinase, imatinibe, que embora seja alvo-específico, cerca de 30% dos pacientes desenvolve resistência ao tratamento. Desta forma, o objetivo deste trabalho foi investigar a associação de variações de nucleotídeo único (SNVs) em genes envolvidos na farmacogenômica do imatinibe com a resistência ao tratamento da LMC com imatinibe. A primeira etapa analisou a relação entre 32 SNVs em genes implicados no processo carcinogênico com a falha terapêutica do imatinibe, utilizando a tecnologia de PCR em tempo real através do QuantStudio™ 12K Flex Real-Time PCR (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Foram genotipados 165 pacientes com LMC e tratados com imatinibe, divididos em dois grupos: pacientes que responderam bem ao tratamento e pacientes que apresentaram falha terapêutica. Os resultados mostraram que os SNVs rs2372536 no gene *ATIC* (p = 0,045; HR = 2,726; 95% IC= 0,9986–7,441) e rs10821936 no *ARID5B* (p = 0,02; HR = 0,4053; IC 95%= 0,1802–0,911) são estatisticamente significantes para esta variável clínica. A segunda etapa consistiu em analisar 30 variações do tipo SNV em genes envolvidos na farmacogenômica do imatinibe com a resistência secundária ao tratamento da LMC, através da tecnologia do Agena Bioscience™ iPlex Assay, que utiliza a técnica de MassArray para a genotipagem do DNA. Nesta fase, foram investigados 129 pacientes divididos entre: pacientes com boa resposta ao tratamento com imatinibe na LMC e pacientes com resistência secundária. Nossos resultados apontaram que pacientes com o genótipo recessivo AA para a variante rs2290573 do gene *ULK3* tem três vezes mais chances de desenvolver resistência ao longo do tempo do que pacientes com outros genótipos (p = 0,019; OR = 3,19; IC 95%= 1,21-8,36). Além disso, realizamos a análise de interação das 30 variantes investigadas e diversas combinações apresentaram resultados estatisticamente significativos para este tipo de resistência. Concluímos que as variantes citadas acima podem ser relevantes para a predição de resposta ao tratamento da LMC com imatinibe e de possíveis resistências ao tratamento, e a sua utilização como biomarcadores poderia auxiliar o manejo terapêutico e a qualidade de vida dos pacientes.

**Palavras-chave:** Leucemia Mieloide Crônica, farmacogenômica, farmacogenética, imatinibe, *ATIC, ARID5B, ULK3,* interações.

**ABSTRACT**

Chronic Myeloid Leukemia (CML) is the abnormal proliferation of the granulocytic lineage, caused by the balanced reciprocal translocation between the long arms of chromosomes 9 and 22 in the q34 and q11 regions and the formation of the Philadelphia (Ph) chromosome. The first-line treatment of CML is performed with the tyrosine-kinase inhibitor, imatinib, which although it is target-specific, about 30% of patients develop resistance to treatment. Thus, the aim of this work was to investigate the association of single nucleotide variations (SNVs) in genes involved in the pharmacogenomics of imatinib with resistance to treatment of CML with imatinib. The first stage analyzed the relationship between 32 SNVs in genes implicated in the carcinogenic process with imatinib therapeutic failure using real-time PCR technology using QuantStudio™ 12K Flex Real-Time PCR (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). A total of 165 patients with CML and treated with imatinib were genotyped, divided into two groups: patients who responded well to treatment and patients who experienced therapeutic failure. The results showed that the SNVs rs2372536 in the ATIC gene ($p = 0.045$; HR = 2.726; 95% CI= 0.9986-7.441) and rs10821936 in ARID5B ($p = 0.02$; HR = 0.4053; 95% CI= 0.1802-0.911) are statistically significant to this clinical variable. The second stage consisted of analyzing 30 SNVs in genes involved in the pharmacogenomics of imatinib with secondary resistance to CML treatment, using the Agena Bioscience™ iPlex Assay technology, which uses the MassArray technique for DNA genotyping. In this phase, 129 patients were investigated divided into: patients with good response to imatinib treatment in CML and patients with secondary resistance. Our results pointed out that patients with the AA recessive genotype for the rs2290573 variant of the ULK3 gene are three times more likely to develop resistance over time than patients with other genotypes ($p = 0.019$; OR = 3.19; 95% CI= 1.21-8.36). In addition, we performed interaction analysis of the 30 variants investigated and several combinations showed statistically significant results for this type of resistance. We conclude that the variants cited above may be relevant for predicting response to treatment of CML with imatinib and possible treatment resistance, and their use as biomarkers could aid therapeutic management and quality of life of patients.

**Keywords:** Chronic Myeloid Leukemia, pharmacogenomics,pharmacogenetics, imatinib, *ATIC, ARID5B, ULK3,* interactions..

## LISTA DE FIGURAS

Figura 1 – Formação do cromossomo Philadelphia........................................................ 12
Figura 2 - Ativação da via molecular de sinalização da proteína BCR-ABL no processo de leucemogênese da LMC........................................................................................ 13
Figura 3 - Número de pacientes tratados no SUS em 2017, de acordo com a faixa etária.......................................................................................................................... 15
Figura 4 - Mecanismo de ação da BCR-ABL e de sua inibição pelo imatinibe............... 17
Figura 5 - Níveis de resposta citogenética e molecular segundo a escala internacional... 20
Figura 6 - Ilustração da fita do domínio da quinase ABL em complexo com imatinibe... 24
Figura 7 - Representação dos genes candidatos envolvidos no metabolismo de imatinibe. 27

## LISTA DE QUADROS

Quadro 1 - Definições de resposta ao tratamento da LMC segundo a National Comprehensive Cancer Network (NCCN)...................................................................... 19
Quadro 2 – Marcos temporais de tratamento para análise de resposta molecular no tratamento da LMC de acordo com a escala internacional (IS). ...................................... 21
Quadro 3- Recomendação de tratamento de acordo com as mutações que conferem resistência no gene *BCR-ABL.* .......................................................................... 26
Quadro 4 - Resumo dos resultados encontrados nos artigos.......................................... 84

## LISTA DE ABREVIATURAS E SIGLAS

ADME - Absorção, distribuição, metabolização e excreção

ANVISA - Agência Nacional de Vigilância Sanitária

ATP - Adenosina trifosfato

ELN - *European Leukemia Net*

FDA - *Food and Drug Administration*

FISH - Hibridação *in situ* por fluorescência

GISTs - Tumores estromais gastrointestinais

HU - Hidroxiuréia

IARC - *International Agency for Research on Cancer*

IMB – Imatinibe

ITQ - Inibidores de tirosina-quinase

LLA - Leucemia linfoblástica aguda

LMC - Leucemia Mieloide Crônica

NCCN - *National Comprehensive Cancer Network*

OMS - Organização Mundial da Saúde

PCR - Reação em Cadeia da Polimerase

Ph- Cromossomo Philadelphia

RCC - Remissão Citogenética Completa

RMM - Resposta Molecular Maior

RM4 - Resposta Molecular com redução de 4 logs

RM4,5 - Resposta Molecular com redução de 4,5 logs

SIA - Sistema de Informação Ambulatorial

SUS - Sistema Público de Saúde

SUMÁRIO

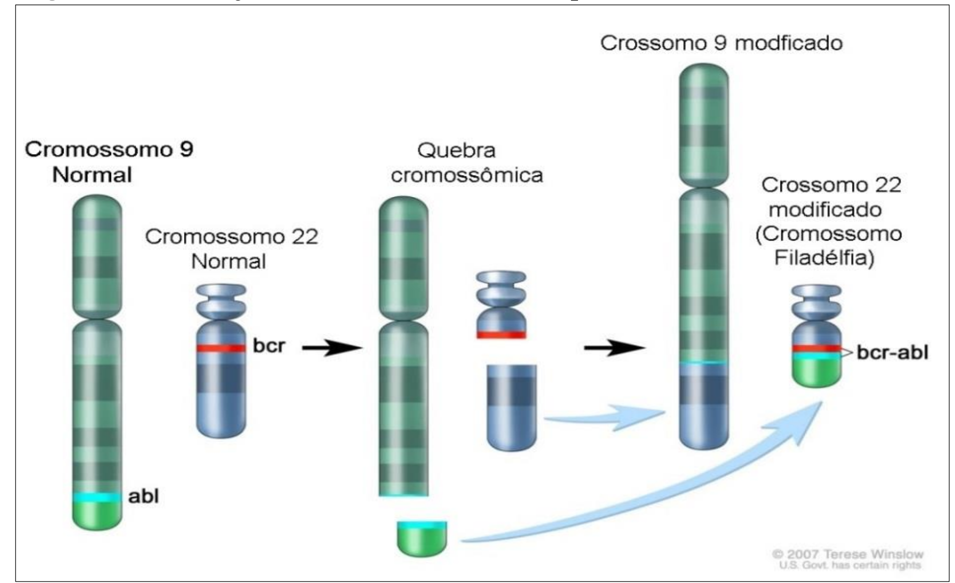
**1. INTRODUÇÃO** ..... 12
**1.1 Leucemia Mieloide Crônica** ..... 12
**1.2 Epidemiologia da Leucemia Mieloide Crônica** ..... 13
**1.3 Diagnóstico da Leucemia Mieloide Crônica** ..... 15
**1.4 Tratamento da Leucemia Mieloide Crônica** ..... 16
**1.5 Resposta ao tratamento** ..... 18
**1.6 Farmacogenômica** ..... 21
**1.7 Resistência ao tratamento** ..... 23
1. 7. 1 Mecanismos dependentes de *BCR-ABL* ..... 23
1. 7. 2 Mecanismos independentes de *BCR-ABL* ..... 26
1.7.2.1 *ABCB1* ..... 27
1. 7. 2. 2. *ABCG2* ..... 28
1. 7. 2. 3. *SLC22A1* ..... 29
1. 7. 2. 4. Outros genes transportadores ..... 30
1. 7. 2. 5. Genes metabolizadores ..... 31
1. 7. 2. 6. Outros genes ..... 32
**1.8 Ancestralidade** ..... 33
**2. JUSTIFICATIVA** ..... 34
**3. OBJETIVOS** ..... 35
**3.1 Objetivo geral** ..... 35
**3.2 Objetivos específicos** ..... 35
**4. ATIVIDADES DE PESQUISA DESENVOLVIDA PELA PROPONENTE DURANTE O DOUTORADO** ..... 36
**CAPÍTULO I: Impact of Variants in the *ATIC* and *ARID5B* Genes on Therapeutic Failure with Imatinib in Patients with Chronic Myeloid Leukemia** ..... 37
**CAPÍTULO II: Alterations in pharmacogenetic genes and their implications for imatinib resistance in CML patients from an admixed population** ..... 50
**5. DISCUSSÃO** ..... 78
**6. CONCLUSÃO** ..... 80
**REFERÊNCIAS** ..... 81
**ANEXO I: Polymorphisms in the *CYP2A6* and *ABCC4* genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population** ..... 90
**ANEXO II - Aprovação do comitê de ética em pesquisa** ..... 100
**ANEXO III - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO** ..... 101

# 1. INTRODUÇÃO

## 1.1 Leucemia Mieloide Crônica

A Leucemia Mieloide Crônica (LMC) é a proliferação anormal da linhagem granulocítica sem a perda de capacidade de diferenciação, ocasionada pela translocação recíproca e equilibrada entre os braços longos dos cromossomos 9 e 22 nas regiões q34 e q11, respectivamente (figura 1). Essa translocação leva à formação do cromossomo Philadelphia (Ph), a principal característica dessa leucemia (PERROTI, 2010; APPERLEY, 2015).

**Figura 1 –** Formação do cromossomo Philadelphia.




Translocação recíproca de regiões dos braços longos do cromossomo 9 e do cromossomo 22 (9;22) (q34; q11). O gene *BCR-ABL* é formado no cromossomo 22, que passa a ser chamado de cromossomo Philadelphia. **Fonte:** Adaptado de National Cancer Institute, 2007

A reorganização destes cromossomos leva à união dos genes *BCR* (breakpoint cluster region) e *ABL* (Abelson tyrosine-protein kinase 1) e formação do gene quimérico *BCR-ABL.* Como consequência, ocorre a expressão de uma proteína tirosina quinase constitutiva, também denominada BCR-ABL. A presença desta proteína mutante tem como resultado a ativação dos alvos de sinalização celular que levam à uma proliferação descontrolada das células mieloides. Pesquisas apontam que, apesar de ser a mais frequente, não é somente a translocação dos cromossomos 9 e 22 que gera a fusão dos genes *BCR-ABL.* Em apenas 5% dos casos, há a formação de translocações complexas envolvendo um terceiro cromossomo (ANKATHIL et al., 2018).

A presença desta proteína explica, virtualmente, todas as características celulares da leucemia: proliferação desordenada, inibição da apoptose, alteração da adesão celular, fatores

de crescimento independentes e diferenciação prejudicada (DALEY; VAN ETTEN; BALTIMORE, 1990; KANG et al., 2016). Os mecanismos que levam a desregulação celular envolvem várias vias de sinalização, como JAK/ STAT, PI3K/AKT, Ras/ MEK, SKP2, FOXO3, mTOR, MAPK e MYC (YANG; FU, 2015; BRAUN; EIDE; DRUKER, 2020). Este processo está representado na Figura 2.

**Figura 2** – Ativação da via molecular de sinalização da proteína BCR-ABL no processo de leucemogênese da Leucemia mieloide crônica

**Fonte:** Adaptado de BRAUN; EIDE; DRUKER, 2020.

## 1.2 Epidemiologia da Leucemia Mieloide Crônica

A LMC representa cerca de 7 - 20% dos casos de leucemias adultas, com incidência de 1 a 2 casos a cada 100.000 pessoas e com 8.430 novos casos por ano em uma perspectiva global (RODRIGUEZ-ABREU; BORDONI; ZUCCA et al., 2007; MAURO et al., 2015; SIEGEL; MILLER; JEMAL, 2020; SHALLI et al., 2020).

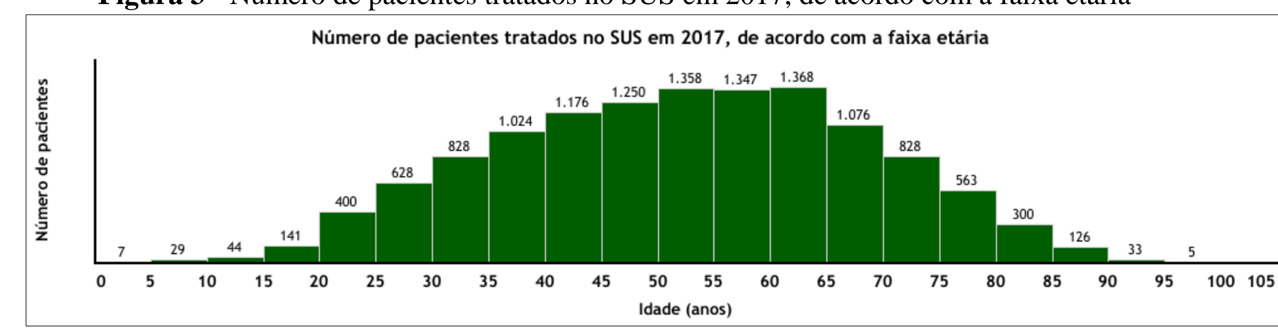
Mendizabal e colaboradores (2016) realizaram um estudo epidemiológico utilizando o registro da base populacional disponibilizada *pela International Agency for Research on Cancer* (IARC) com 33.690 pacientes com LMC envolvendo 60 países e os cinco continentes. Segundo este estudo, do total de pacientes investigados 57,2% dos diagnósticos eram do sexo masculino revelando a maior prevalência da LMC em homens do que em mulheres. Este fato é relatado pela literatura que aponta a incidência da LMC maior em homens do que em mulheres com uma razão de 2,2/ homem- 1,4/ mulher e isto pode estar relacionado com os hormônios femininos mas ainda não foi completamente elucidado (BERGER et al., 2005; LIN et al., 2020).

Além disso, os estudos apontam que há baixa incidência de LMC em crianças e que esta taxa vai crescendo com a idade, chegando ao ápice na faixa de 75-80 anos e depois caindo drasticamente, apontando assim a idade como um fator de risco para a LMC. Esta distribuição provavelmente está associada com a diminuição na função hematopoiética do organismo que é diretamente associada com a leucemogênese (HAAN; LAZARE et al., 2018; NING et al., 2020; LIN et al., 2020). Ainda segundo Mendizabal et al. (2016), a mediana de idade ao diagnóstico apresenta variações entre as diferentes regiões geográficas do planeta. As menores idades estão na África e na Ásia (47 anos), enquanto as maiores estão na Oceania (72 anos).

A incidência também sofre mudanças de acordo com a localidade, a menor taxa é na Ásia (0,55 por 100 mil/ habitantes) e a maior nos países da Oceania (1,78 por 100 mil/ habitantes). Essas diferenças entre as regiões demonstram que podem existir fatores ambientais e econômicos que influenciam a incidência da LMC. O estudo também levanta a possibilidade de predisposição em relação ao grupo étnico, mas que precisa ser melhor investigada (HÖGLUND; SANDIN; SIMONSSON, 2015, MENDIZABAL; YOUNES; LEVINE, 2016).

No que diz respeito ao Brasil, existem poucas informações disponíveis acerca das taxas de incidência e prevalência da LMC. No entanto, em um levantamento feito no Sistema de Informação Ambulatorial do Sistema Único de Saúde (SIA/SUS) constatou-se que até o ano de 2017, o número de pacientes com LMC tratados no SUS era de 19.733, representando uma taxa de 9,7 pacientes por 100 mil habitantes. Ao comparar os dados dos anos 2017 com o número de pacientes tratados em 2009 (6.015), podemos notar que a taxa de pacientes tratados cresceu cerca de 13,2% ao ano. Ainda de acordo com estes dados, 55% dos pacientes atendidos pelo SUS são do gênero masculino e 45% são do gênero feminino, e maior parte dos pacientes estão concentrados na faixa entre 60-65 anos. A distribuição completa destas informações pode ser visualizada na figura 3 (MELO, 2019).

**Figura 3 -** Número de pacientes tratados no SUS em 2017, de acordo com a faixa etária




**Fonte:** Observatório de Oncologia, 2019.

### 1.3 Diagnóstico da Leucemia Mieloide Crônica

O diagnóstico da LMC é realizado por várias etapas, desde o exame clínico até exames laboratoriais específicos sobre os quais serão descritos abaixo.

O diagnóstico clínico é dado pela anamnese e exame físico realizado pelo médico, com foco na palpação do baço e do fígado que normalmente aumentam de tamanho na LMC. No entanto, de acordo com Hocchaus et al. (2017) em um estudo feito na Europa, cerca de 50% dos pacientes diagnosticados são assintomáticos. Quando presentes, os sinais mais comuns são fadiga, perda de peso, mal-estar e sensação de plenitude gástrica, decorrentes da anemia e da esplenomegalia. Outros sintomas que aparecem de maneira mais rara são sangramento (devido a disfunções na plaqueta ou plaquetopenia), trombose (como consequência da trombocitose), artrite (por níveis elevados de ácido úrico e consequente acúmulo nas extremidades), hemorragias na retina e ulceração gastrointestinal superior (por níveis elevados de histamina associados à basofilia) (BAIN, 2005; HOFFMAN et al., 2017).

Nos pacientes assintomáticos, o principal exame que revela a presença da LMC é o hemograma, já que ao realizar exames de rotina nota-se as alterações e então são encaminhados à um serviço hematológico especializado. Geralmente, no hemograma encontra-se leucocitose e eventualmente trombocitose. No entanto, a confirmação do diagnóstico só é feita pela detecção da presença do cromossomo Philadelphia através do exame citogenético ou molecular (BACCARANI; DREYLING, 2010).

O exame citogenético é realizado a partir do mielograma, onde é feita a aspiração da medula óssea para análise por bandeamento G ou pela técnica de hibridação *in situ* por fluorescência (FISH) para a identificação do cromossomo Philadelphia. Esta modalidade de diagnóstico é importante pois é possível analisar o nível de fibrose, a morfologia das células e fazer a diferenciação das células blásticas e dos basófilos a fim de diferenciar entre a fase blástica e a acelerada (JABBOUR; KANTARIJAN, 2016; HOCHHAUS et al., 2020). No

entanto, atualmente, este tipo de diagnóstico é pouco utilizado devido à ascensão de ferramentas de diagnóstico que utilizam a biologia molecular.

Assim, o principal exame utilizado para o diagnóstico é a Reação em Cadeia da Polimerase (do inglês, *Polymerase Chain Reaction* – PCR) do gene *BCR-ABL*. Esta técnica de biologia molecular identifica e amplifica as cópias de DNA deste gene por meio de sondas específicas de detecção e permite o diagnóstico preciso deste tipo de leucemia. A técnica apresenta como vantagens: i) ser minimamente invasiva, pois é realizada com amostras de sangue periférico coletado por punção venosa; e ii) ser altamente sensível e precisa, sendo utilizada também para acompanhamento da resposta ao tratamento e detecção de doença residual mínima (JABBOUR; KANTARIJAN, 2016; HOCHHAUS et al., 2020).

Atualmente, o exame de PCR do gene *BCR-ABL* é o critério utilizado pelo Ministério da Saúde do Brasil para a liberação medicamento utilizado como primeira linha para o tratamento alvo-específico da LMC no país e fornecido pelo SUS, o inibidor de tirosina quinase: imatinibe (BRASIL, 2008).

### 1.4 Tratamento da Leucemia Mieloide Crônica

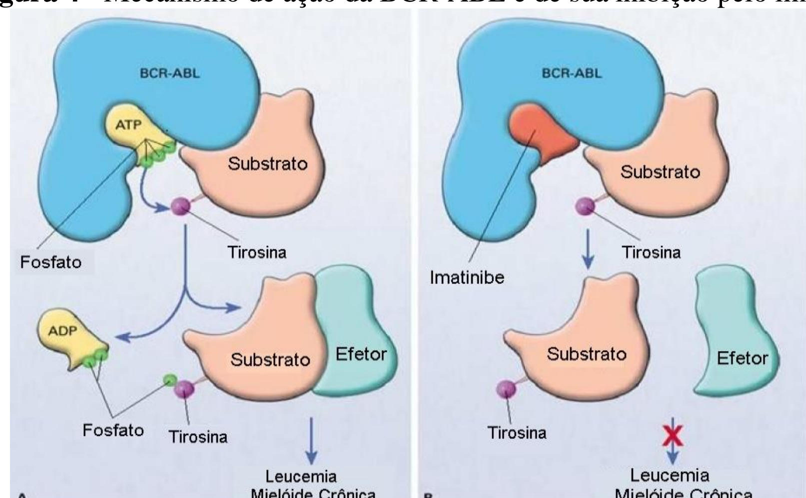
O tratamento da LMC passou por evoluções ao longo dos anos: a Hidroxiuréia (HU) foi utilizada como o tratamento padrão durante muito tempo. Este medicamento é um agente citostático que controla a leucometria e assim reduz os sintomas associados a essa condição. E por isso, a HU continua sendo utilizada no início do tratamento até que o tratamento com inibidores de tirosina quinase seja iniciado (VARDIMAN, 2008). Outro medicamento amplamente utilizado foi o Interferon-alfa que apresentava resultados efetivos nos quadros clínicos e laboratoriais dos pacientes com 80% dos pacientes alcançando a resposta hematológica completa e 58% alcançando a resposta citogenética (FRAZER; IRVINE; MCMULLIN, 2007; HAND et al., 2014).

No entanto, nenhum destes medicamentos era de uso exclusivo para LMC e por isso existia altas taxas de mortalidade associada a este tipo de leucemia (FAUSEL, 2007).

A partir do final do ano 2000, os inibidores de tirosina-quinase (ITQ) começaram a figurar no tratamento da LMC como um fármaco alvo-específico que aumentou significativamente a taxa de resposta ao tratamento e sobrevida dos pacientes (DRUKER et al., 2006). Esta evolução foi possível devido ao mecanismo de ação dos ITQs que se ligam ao receptor de ATP (adenosina trifosfato) da proteína BCR-ABL, não permitindo a ligação do grupo fosfato da molécula de ATP e mantendo a proteína inativada (Figura 4). Desta maneira, toda a cascata de sinalização que seria ativada por ela deixa de existir e as células leucêmicas

não se dividem, inibindo o desenvolvimento da neoplasia (FAUSEL, 2007; ANKATHIL et al., 2018).

**Figura 4 -** Mecanismo de ação da BCR-ABL e de sua inibição pelo imatinibe




A. Mostra a oncoproteína BCR-ABL com a molécula de adenosina trifosfato (ATP) em uma bolsa de quinase. O substrato é ativado pela fosforilação dos resíduos de tirosina. B. A ação de BCR-ABL é inibida quando o imatinibe ocupa o lugar de ligação do ATP, impedindo a fosforilação do seu substrato. **Fonte:** Adaptado de GOLDMAN; MELO (2003).

O primeiro ITQ a ser fabricado e disponibilizado para o tratamento da LMC foi o imatinibe (IMB). O estudo IRIS (International Randomized Study of Interferon and STI571) foi a principal evidência de eficácia no imatinibe na LMC. Este estudo acompanhou por 60 meses dois grupos de pacientes, um grupo foi tratado com imatinibe e outro com interferon alfa e citarabina. Ao comparar as respostas dos pacientes ao final do estudo, notou-se que 83% dos pacientes tratados com o imatinibe alcançou maior sobrevida livre de eventos, 93% dos pacientes não tiveram progressão da doença e houve o aumento de 95% da sobrevida global dos pacientes. Além disso, a taxa de resposta hematológica foi de 98%, a taxa de resposta molecular foi 92% e a de resposta citogenética foi de 87% (DRUKER et al., 2006).

O imatinibe (STI-571, Glivec®) é um composto 2-fenilamino-pirimidina que inibe a autofosforilação da proteína BCR-ABL por ser um competidor inibitório de ATP. Este medicamento é utilizado, principalmente, para o tratamento da LMC, de tumores estromais gastrointestinais (GISTs) e de casos de Leucemia linfoblástica aguda com a presença do cromossomo Philadelphia (LLA Ph+) (NOVARTIS, 2017).

Como citado anteriormente, este foi o primeiro ITQ que mostrou eficiência no tratamento da LMC, foi aprovado e lançado pela *Food and Drug Administration* (FDA -Estados Unidos da América) e pela Agência Nacional de Vigilância Sanitária (Anvisa – Brasil) em 2001

e logo passou a ser utilizado como tratamento de primeira linha da LMC. Atualmente, a recomendação de administração é 400mg/ dia de imatinibe para pacientes na fase crônica, com possibilidade de ajuste para 300mg/dia se não há boa tolerância ao medicamento e a resposta continua eficaz. Já para pacientes na fase aguda, a dose recomendada é de 800mg/ dia (NOVARTIS, 2017; WALLER, 2018).

Além de inibir a proteína BCR-ABL, o imatinibe também funciona como inibidor de outras vias de sinalização, como as ativadas por receptor do fator de crescimento derivado de plaquetas (PDGFR), c-Kit (membro do tipo III de receptores quinases), MAPK (Mitogen Activated Protein Kinases) e PI3K/AKT (Phosphatidyl inositol 3 kinase) e assim age por diversos meios para bloquear a divisão celular (YANG; FU, 2015).

O imatinibe produz eventos adversos leves e moderados, sendo os mais frequentes: edema, dores ósseas ou articulares, alterações enzimáticas no fígado, citopenia de algumas das linhagens sanguíneas ou pancitopenias (WALLER, 2018). E na maioria dos casos a suspensão do fármaco é suficiente para que os eventos adversos deixem de existir e, geralmente, ele é reintroduzido quando os sintomas desaparecem. Se os eventos adversos forem graves ou houver intolerância ao medicamento, é necessário a descontinuidade do imatinibe e por vezes a troca do medicamento (HOCHHAUS et al., 2015; SHAH, 2019).

Outros medicamentos utilizados no tratamento da LMC e que são utilizados como segunda e terceira linha de tratamento são os inibidores de tirosina quinase: dasatinibe, nilotinibe, bosutinibe e ponatinibe. Estes medicamentos são utilizados quando há intolerância ou perda de resposta ao tratamento com o fármaco da fase anterior. Eles têm como vantagem serem eficazes contra as mutações no gene *BCR-ABL,* que conferem resistência ao tratamento contra as quais o imatinibe é ineficiente, pois possuem mecanismos de ação diferente do fármaco de primeira linha e como desvantagem o fato de desencadearem efeitos adversos mais severos que os do imatinibe (LINDAUER; HOCHHAUS, 2018; SACHA; SAGLIO, 2018).

O transplante de medula óssea é outra alternativa de tratamento para LMC, inclusive é único tratamento considerado como curativo. No entanto, existem vários riscos associados a este procedimento e por isso apenas é recomendado quando o paciente apresenta resistência a variados tipos de inibidores de tirosina-quinase ou o paciente está em fase acelerada ou blástica e não tem resposta aos ITQs (HOCHHAUS et al., 2020).

### 1.5 Resposta ao tratamento

A resposta ao tratamento com imatinibe é monitorada constantemente para que possíveis resistências possam ser detectadas precocemente. Assim são realizadas avaliações

hematológica, citogenética e/ou molecular desde o início da terapia e em períodos regulares de tempo. Estas avaliações são baseadas nos critérios estabelecidos National Comprehensive Cancer Network (NCCN) e reconhecidos pela Organização Mundial da Saúde (OMS) como descritos no quadro 1:

**Quadro 1** - Definições de resposta ao tratamento da LMC segundo a *National Comprehensive Cancer Network* (NCCN)

| Resposta Hematológica | Resposta Citogenética | Resposta Molecular |
|---|---|---|
| Completa normalização da contagem das células sanguíneas com leucócitos < $10x10^9$/L;<br>Plaquetas < 450 x $10^9$/L;<br>Ausência de precursores no sangue (mielócitos, promielócitos ou blastos);<br>Ausência de sinais e sintomas e desaparecimento da esplenomegalia. | **Completa:** 0% $Ph^+$<br>**Maior:** 1-35% $Ph^+$<br>**Parcial:** 36-65% $Ph^+$<br>**Mínima:** 66-95% $Ph^+$<br>**Ausência:**> 95% $Ph^+$ | **Ótima:** BCR-ABL1 (IS[1]) ≤10% em 3 e 6 meses<br>**Maior:** BCR-ABL1 (IS[1]) ≤ 0,1% (redução ≥ 3-log)<br>**Completa:** BCR-ABL negativo e/ou indetectável por PCR; |

[1] Escala internacional (*International scale* – IS). **Fonte:** SHAH, 2019.

Atualmente, o exame de citogenética é utilizado com menos frequência e deixou de ser o principal parâmetro para avaliar a eficácia do tratamento pois é menos aplicável devido a demora prática que a técnica requer do que o exame molecular. Todavia, é útil para a identificação de pacientes com translocações atípicas e raras que não são identificadas pelo exame de PCR do gene *BCR-ABL* (HOCHHAUS et al., 2020).

Assim, o monitoramento da resposta ao imatinibe é feito principalmente pelo exame de PCR do gene *BCR-ABL,* pois é um método minimamente invasivo e mais específico. A resposta molecular é estabelecida de acordo com a Escala Internacional (*Internacional Scale* – IS) a partir da razão entre os transcritos de *BCR-ABL* e os transcritos de *ABL* (a expressão do gene sem mutação) e é expressa como a porcentagem de *BCR-ABL* em escala de log onde, quando o paciente chega a um nível de expressão de: 1%, 0,1%, 0,01%, 0,0032% e 0,001% corresponde a redução de 2, 3, 4, 4,5 e 5 logs, respectivamente. A linha de base utilizada aqui é a mesma do estudo IRIS citado anteriormente (CROSS et al., 2012; HOCHHAUS et al., 2020).

Quando o nível de transcrição do *BCR-ABL* é <1% equivale a remissão citogenética completa (RCC), já quando este nível está em ≤0,1% de cópias do gene modificado é definido

como resposta molecular maior (RMM) ou RM3 (redução de 3 logs). Um nível de ≤0,01% de transcritos é classificado como indetectável e corresponde a RM4 (resposta molecular com redução de 4 logs). O nível de transcritos de *BCR-ABL* ≤0,0032% é definido como RM4,5 (resposta molecular com redução de 4,5 logs). Esta distribuição pode ser visualizada na figura 5 (CROSS, 2015; HOCHHAUS et al., 2020).

**Figura 5 -** Níveis de resposta citogenética e molecular segundo a escala internacional

RCM: Resposta citogenética maior; RCC: Resposta citogenética completa; RM: Resposta molecular; RM4: Resposta molecular com redução de 4 logs; RM4,5: Resposta molecular com redução de 4,5 logs; RM5: Resposta molecular com redução de 5 logs. **Fonte:** MAHON; ETIENNE (2014)

Quanto à frequência de monitoramento das avaliações, é recomendado que sejam realizados hemogramas a cada 2 semanas até que se alcance a resposta hematológica completa. O monitoramento molecular dos níveis de transcritos de *BCR-ABL* deve ser feito nos marcos temporais de 3, 6 e 12 meses (HOCHHAUS et al., 2020).

De acordo com os resultados obtidos, a conduta é determinada para o paciente: o tratamento deve ser continuado quando a resposta é classificada como ótima, deve ser modificado quando há falha ou resistência no tratamento ou ainda ser continuado com restrições dependendo das características de comorbidades e tolerância ao medicamento (em alerta). A descrição completa da análise de resposta ao tratamento de acordo com os marcos temporais está no quadro 2 (HOCHHAUS et al., 2020).

**Quadro 2** – Marcos temporais de tratamento para análise de resposta molecular no tratamento da LMC de acordo com a escala internacional (IS).

| Resposta | Ótima | Alerta | Falha |
|---|---|---|---|
| Linha de base | NA | Alto risco de outras anormalidades cromossômicas | NA[1] |
| 3 meses | ≤10% | >10% | >10% se confirmado entre 1-3 meses |
| 6 meses | ≤1% | >1-10% | >10% |
| 12 meses | ≤0,1% | >0,1-1% | >1% |
| Em qualquer tempo | ≤0,1% | >0,1-1%, perda de ≤0,1% (RMM)[2] | >1%, mutações resistentes, alto risco de translocações diferentes. |

[1]NA- não aplicável; [2] Perda de MMR (*BCR-ABL*>0,1%) indica falha depois de tratamento livre de remissão. **Fonte:** HOCHHAUS et al. (2020)

A mudança de tratamento passa a ser considerada se a RMM não é alcançada entre 36 e 48 meses. Para pacientes que estão livres de remissão, em qualquer tempo de tratamento, assume-se a marca de *BCR-ABL* ≤0,01% (RM4) para resposta ótima (HOCHHAUS et al., 2020).

A introdução de uma segunda linha de tratamento é recomendada quando a resposta à primeira linha não é evidente, há toxicidade ou intolerância ao fármaco a ponto de ser necessário interrupção e/ou redução de dose. Nos casos de alerta, a mudança é opcional e ao critério médico. Além disso, independente da causa da mudança, o próximo ITQ a ser escolhido deve ser levado em consideração fatores como idade e comorbidades (HOCHHAUS et al., 2020). E especificamente para o Brasil, as diretrizes do Ministério da Saúde preconizam que são necessárias 3 a 4 avaliações antes de efetuar a troca da medicação (BRASIL, 2021).

### 1.6 Farmacogenômica

Na vivência clínica é conhecido que existe variabilidade na resposta dos pacientes aos tratamentos utilizados: doses definidas como padrão são eficazes em alguns pacientes, enquanto podem ser ineficazes ou causar efeitos adversos em outros. Diversos fatores como: gênero, idade, etnia, fumo, etilismo e variações genéticas podem influenciar esta variação de respostas (SADEE; DAÍ, 2005; RODEN et al., 2019).

Dentro deste aspecto, a farmacogenômica investiga como as características genéticas de um indivíduo podem influenciar a resposta aos medicamentos, já que tanto a farmacocinética quanto a farmacodinâmica dos fármacos podem ser influenciadas por estas características (HERTZ; RAE, 2015; NAKAGAWA; FUJITA, 2018).

A abordagem tradicional da farmacogenômica baseia-se em estudar variantes de DNA em genes que participam ativamente da absorção, distribuição, metabolização e excreção

(ADME) de fármacos e que podem levar à uma variabilidade de resposta aos medicamentos (WEINSHILBOUM; WANG, 2006; GUO et al., 2019).

A aplicação da farmacogenômica na área oncológica é um processo complexo, porque envolve o difícil manejo clínico do tratamento aplicado a dois genomas: o do hospedeiro com as células sem câncer (representado por mutações germinativas) e o genoma das células com o câncer (representado por mutações somáticas), que apresenta um papel crítico na resposta antineoplásica (HERTZ; RAE, 2015; RODRÍGUEZ-VICENTE et al., 2016; NAKAGAWA; FUJITA, 2018).

A busca por novas estratégias terapêuticas, menos tóxicas e menos invasivas, evidencia a interface entre a pesquisa básica e o atendimento clínico. A evolução deste campo de pesquisa é uma tendência global, e tem como objetivo associar o desenvolvimento de esquemas terapêuticos ao perfil genético dos pacientes, com a finalidade de oferecer uma terapia individualizada que possa maximizar a eficácia dos fármacos e minimizar os efeitos adversos associados a eles (GUO et al., 2019).

Dessa forma, os rápidos avanços na genômica funcional têm permitido estabelecer novas estratégias de terapia, que permitem oferecer ao paciente um tratamento personalizado com a escolha do fármaco ideal, na dose adequada para cada indivíduo, tomando por base os perfis moleculares do paciente tanto de células normais como de células tumorais. (RODRÍGUEZ-ANTONA; TARON, 2015; RODRÍGUEZ-VICENTE et al., 2016; BUKOWSKI; KCIUK; KONTEK, 2020).

Nos últimos anos, muito se investigou sobre a aplicação desta terapia individualizada e alguns estudos obtiveram resultados significativos e são aplicados na prática clínica. Este é o caso das variantes genéticas do gene *HER2*, também conhecido como *ERBB2 (Erb-B2 receptor tyrosine kinase 2)* e o tratamento com transtuzumabe em câncer de mama, bem como o de variantes genéticas do gene *EGFR* (Epidermal Growth Factor Receptor) e o tratamento com gefitinibe, em câncer de pulmão de não pequenas células (LOIBL; GIANNI, 2017; TU et al., 2017).

Outro exemplo que pode ser citado é o próprio tratamento com imatinibe já que as agências de regulação de fármacos (como a FDA e a *European Medicine Agency* – EMA) de diversos países preconizam que antes do início do tratamento seja feita a identificação do gene *BCR-ABL* para os pacientes de LMC e dos genes *FIP1L1*, *KIT*, *PDGFRA* e *PDGFRB* para pacientes com GIST. Para todos os exemplos citados Assim, como nos outros exemplos citados, é essencial o exame molecular antes do tratamento ser iniciado (NOVARTIS, 2017).

No Brasil, o exame de PCR do gene *BCR-ABL* é fornecido pelo SUS tanto para diagnóstico da LMC quanto para o monitoramento de resposta ao longo do tratamento. No estado do Pará, este exame está disponível o Laboratório de Biologia Molecular (localizado no Hospital Ophir Loyola) que atende os pacientes da região e é pioneiro no estabelecimento deste serviço na região Norte do Brasil.

**1.7 Resistência ao tratamento**

Estudar e tentar compreender as causas e mecanismos de resistência da LMC aos ITQs é um passo importante para oferecer um tratamento mais eficaz aos pacientes. Pois, ainda que o imatinibe seja um fármaco alvo-específico cerca de 30% dos pacientes não respondem bem ao tratamento, estabelecendo um paradigma nesta terapia (YANG; FU, 2015; DE SANTIS et al., 2022)

A resistência ao imatinibe pode ser definida como primária ou secundária, a depender do momento em que ela se apresenta. É classificada como primária quando há a falta de resposta satisfatória, de acordo com os critérios estabelecidos pela OMS, desde o início do tratamento e a secundária é caracterizada quando há uma recaída da doença depois de uma resposta inicial benéfica à terapia (BACCARANI et al., 2013; DE SANTIS et al., 2022).

Os mecanismos de resistência implicados nesse processo têm sido investigados e, atualmente, são divididos entre mecanismos dependentes de *BCR-ABL* e mecanismos independentes de *BCR-ABL* (BIXBY; TALPAZ, 2009; ANKATHIL et al., 2018; DE SANTIS et al., 2022).

1. 7. 1 Mecanismos dependentes de *BCR-ABL*

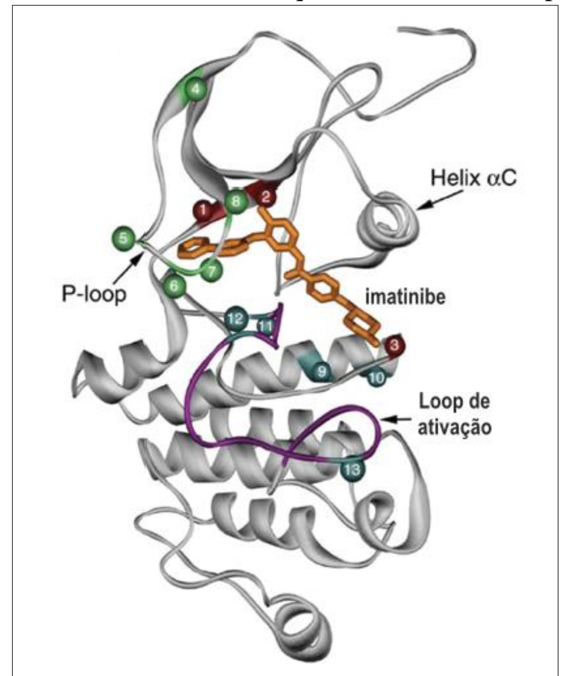
As principais causas apontadas como propiciadoras de resistência ao imatinibe pelo mecanismo dependente de *BCR-ABL* são: a super expressão da proteína BCR-ABL, como consequência da amplificação do seu gene correspondente; mutações no domínio tirosina-quinase do gene *BCR/ABL*, que estão altamente associadas com casos de recaída da doença; e a presença do cromossomo Philadelfia duplo, que conduz rapidamente o quadro clínico à fase blástica da doença (BIXBY; TALPAZ, 2009; ANKATHIL et al., 2018; DAVULCU et al., 2022).

As mutações mais frequentes no domínio tirosina-quinase são as que levam à alteração da conformação da proteína BCR-ABL. A alteração dos aminoácidos na região do sítio de

ligação aos inibidores de tirosina quinase impede que haja uma ligação efetiva, o que interfere na sensibilidade das células ao fármaco (SRIVASTAVA; DUTT, 2013; LINEV et al., 2018).

Estes tipos de mutações se concentram principalmente em quatro regiões do domínio ABL-tirosina quinase, a saber: 1) Loop de ligação do grupo fosfato (P-loop) que normalmente envolve um grupo fosfato no fundo da bolsa de ligação; 2) o loop de ativação (A-loop) que é essencial para a conformação da quinase, 3) os resíduos *gatekeepers* que estão diretamente em contato com o imatinibe e 4) outros resíduos localizados mais abaixo no domínio catalítico que participam na estabilização do A-loop em certa conformação. A organização da proteína BCR-ABL pode ser vista na figura 6 (JOHANSSEN, 2006; LINEV et al., 2018).

**Figura 6 -** Ilustração da fita do domínio da quinase ABL em complexo com imatinibe.




O imatinibe é representado em amarelo, e os números representam resíduos de aminoácidos envolvidos no processo de resistência. Esferas vermelhas (1-3) simbolizam mutações que afetam diretamente a ligação do imatinibe ao medicamento, as esferas verdes (4-8) são mutações P-loop e as esferas azuis (9-13) são mutações próximas ao loop de ativação (loop A). Os resíduos de aminoácidos são: 1 F317L; 2 T315I; 3 F359; 4 M244; 5 G250; 6 Q252; 7 Y253; 8 E255; 9 M351; 10 E355, 11 V379; 12 L387; 13 H396.8. **Fonte:** Adaptado de LINEV et al., 2018.

A mais relevante dessas alterações é a T315I pois confere resistência a variados medicamentos, incluindo o imatinibe (LINEV et al., 2018). Este ponto é importante pois o aminoácido T315 é essencial para a ligação de hidrogênio entre o imatinibe e a proteína no P-loop; assim, a mudança pontual de treonina 315 para isoleucina (T315I) não permite a ligação do hidrogênio e consequentemente não permite a ligação do imatinibe. Além disso, a mutação T315I induz uma mudança conformacional em vários resíduos de aminoácidos da proteína, que

são importantes para a ligação do imatinibe com a BCR-ABL (o efeito alostérico), assim a T315I produz resistência ao imatinibe mais fortemente do que outras mutações pontuais (SHAH et al., 2004; ALI, 2016; LINEV et al., 2018).

O primeiro estudo que investigou mecanismos de resistência ao imatinibe, identificou a mutação T315I decorrente da substituição de C para T na base 944 no gene *ABL* em metade dos pacientes avaliados (GORRE et al., 2001). A literatura também aponta que a taxa dessa mutação é maior em pacientes que alcançaram a fase aguda ou a crise blástica (NATH; WANG; HUANG, 2017).

Outras importantes mutações que conferem resistência aos ITQs estão localizadas na região P-loop, esta região também é conhecida como sítio de ajuste induzido devido a mudança conformacional que é causada ao ocorrer a ligação do imatinibe (KIMURA; ANDO; KOJIMA, 2014). O ajuste induzido permite que o imatinibe estabeleça uma ligação de hidrogênio com a tirosina 253 (Y253) que é intensificada por outros aminoácidos hidrofóbicos ao seu redor. Portanto, as mutações pontuais em Y253 incluindo Y253F e Y253H interferem na ligação do imatinibe. Ainda podemos citar as mutações E255K, F359V e M315T que também ocorrem no P-loop (JABBOUR et al., 2006; KAYASTHA et al., 2017).

E a presença de mutações em diferentes regiões do domínio quinase da proteína leva a diferentes níveis de resistência. Ainda não foi totalmente esclarecido se as mutações que conferem resistência ao tratamento com ITQs surgem ao longo do tratamento ou se são mutações germinativas pré-tratamento (ANKATHIL et al., 2018).

A recomendação da *European Leukemia Net* (ELN) é que a genotipagem para identificação de mutações no gene *BCR-ABL* é mais benéfica para pacientes que apresentam resistência primária ou secundária ao ITQ e não antes de iniciar o tratamento. Assim, a análise da mutação no *BCR-ABL* é um componente importante no monitoramento da doença em pacientes com resistência ao tratamento pois auxilia a identificar que a causa da resistência foram estas mutações e não outros motivos (SOVERINI et al., 2011; ANKATHIL et al., 2018).

Assim, segundo as recomendações da ELN em caso de falha ou resistência ao ITQ de primeira linha é mandatório realizar a troca de medicamento e ser investigado a presença de mutações no domínio quinase da BCR-ABL. Para as mutações mais frequentes (T315I, V299L, T315A, F317L / V / I / C, Y253H, E255K / V e F359V / C / I), os estudos apontam que quando estas mutações estão presentes o imatinibe não é mais recomendado (SRIVASTAVA; DUTT, 2013; SOVERINI et al., 2011; KAYASTHA et al., 2017; LINEV et al., 2018).

A análise dessas mutações específicas deve ser usada para escolher o tipo de ITQ que será utilizado como segunda linha de tratamento, de acordo com as recomendações da ELN. A

detecção dos tipos de mutações deve ser acompanhada por um algoritmo de tratamento específico (quadro 3).

**Quadro 3 -** Recomendação de tratamento de acordo com as mutações que conferem resistência no gene *BCR-ABL*.

| Mutação | Tratamento recomendado |
|---|---|
| T315I | Ponatinibe |
| F317L/V/I/C, T315A | Nilotinibe, bosutinibe ou ponatinibe |
| V299L | Nilotinibe ou ponatinibe |
| Y253H, E255V/K, F359V/I/C | Dasatinibe, bosutinibe, ou ponatinibe |

**Fonte:** HOCHHAUS et al. (2020).

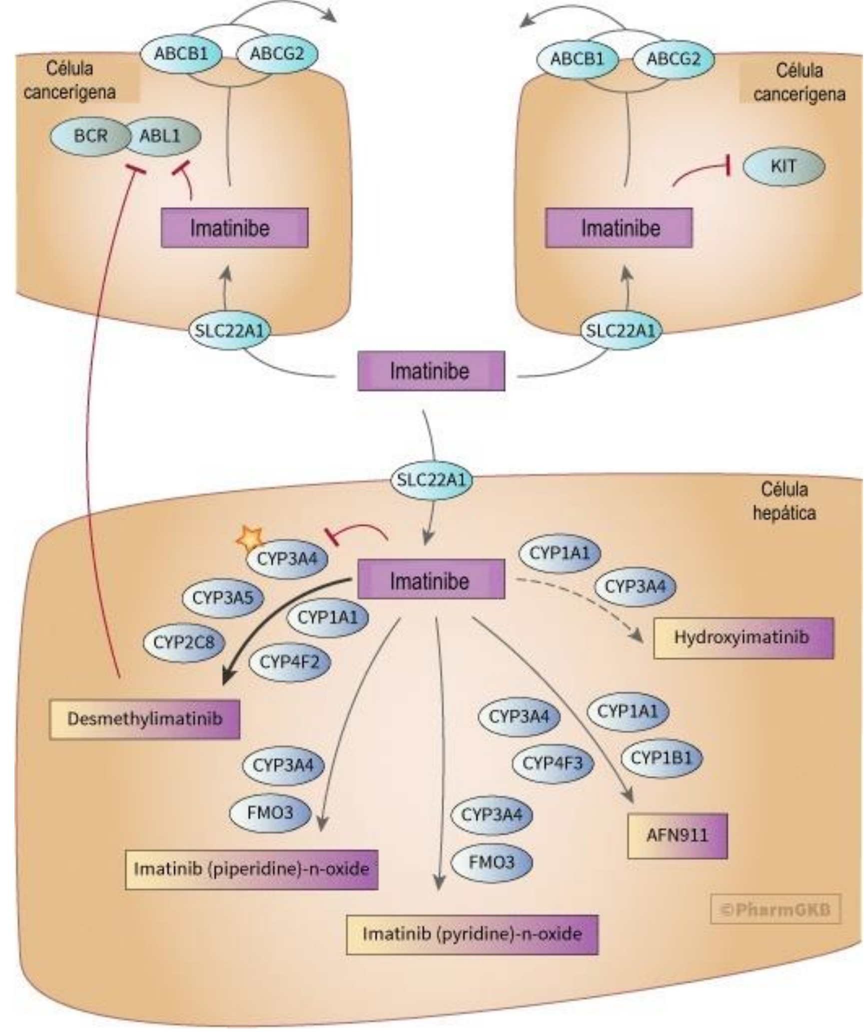
1. 7. 2 Mecanismos independentes de *BCR-ABL*

Ainda que as mutações dependentes de *BCR-ABL* sejam muito relevantes, elas não podem explicar de 30-50% dos casos de resistência ao imatinibe (ANKATHIL et al., 2018). Assim, destaca-se a importância dos mecanismos de resistência não dependentes de BCR-ABL que envolvem uma extensa gama de mecanismos, incluindo alterações genéticas e epigenéticas. Mutações em genes que afetam a farmacocinética, a farmacodinâmica e a biodisponibilidade do fármaco podem diminuir a concentração do medicamento disponível para atuar na célula e colaborar para a resistência ao tratamento (MILOJKOVIC; APPERLEY, 2009).

A metabolização do imatinibe é realizada pelas enzimas codificadas pelos genes da família CYP (*Cytochrome P450 Family*), incluindo os genes *CYP3A4*, *CYP3A5*, *CYP2C8*, *CYP1A2*, *CYP2C9* e *CYP2C19*, e acontece principalmente no fígado (WHIRL-CARRILLO et al., 2012).

O transporte do imatinibe para dentro das células do fígado é, em sua maioria realizado pela proteína OCT1 (*organic cation transporter 1*), expressa pelo gene *SLC22A1 (Solute Carrier Family 22 Member 1)*, que está presente também nas células intestinais e regula o influxo do imatinibe (WANG et al, 2008). Já o efluxo é mediado pelas proteínas P-Gp (*P-glycoprotein*), expressa pelo gene *ABCB1*(também conhecido como *MDR1, Multi-Drug Resistance Gene 1),* presente no intestino, fígado e túbulos renais proximais, e BRCP (*Breast Cancer Resistance Protein*), codificada pelo *ABCG2* (ATP-binding cassette super-family G member 2), também expressa no intestino e no fígado, que influenciam a absorção e a disposição de diversas substâncias nestes órgãos (AU et al., 2014). A via metabólica do imatinibe pode ser visualizada na figura 7.

**Figura 7 -** Representação dos genes candidatos envolvidos no metabolismo de imatinibe.




**Fonte:** Disponível em: https://www.pharmgkb.org/pathway/PA164713427

Diante disso, diversos estudos apontam que a atividade das proteínas transportadoras e metabolizadoras e de ligação do fármaco ao seu receptor pode influenciar a farmacodinâmica e a farmacocinética no organismo e modular a resposta do paciente ao tratamento (SHINOHARA et al., 2013; POLLILO et al., 2015; ANKATHIL et al., 2018). Desta maneira, nas seguintes seções destacaremos os principais genes investigados neste trabalho, destacando estes e suas respectivas proteínas envolvidas na via metabólica do imatinibe e no processo de resistência a ele.

1.7.2.1 *ABCB1*

O gene *ABCB*1 (*MDR1, Multi-Drug Resistance Gene 1*) codifica a proteína glicoproteína- P (P-gp), cuja superexpressão tem sido frequentemente relacionada à resistência

a quimioterapias devido à sua capacidade de efluxo das drogas citotóxicas (WIDMER, et al. 2003; STROMSKAYA, et al. 2011; PENG, et al. 2012).

Galimberti et al. (2005) evidenciaram que o imatinibe é substrato de P-gp e que expressão do gene *ABCB1* influencia a eficiência do fármaco no tratamento da LMC. Eles verificaram que pacientes com LMC que não alcançaram a resposta citogenética completa tinham níveis mais elevados de expressão de *ABCB1* do que os demais pacientes e que havia uma correlação direta entre os níveis de transcrição de *BCR-ABL* e *ABCB1* nestes pacientes.

Outros estudos na literatura corroboram com tais descobertas, visto que o transportador *ABCB1* é super expresso em células de pacientes na fase blástica, o que implica na eficácia reduzida da quimioterapia nas fases mais avançadas, assim como a resistência ao imatinibe (KUWAZURU, et al. 1990; MAHON, et al. 2003, ZU, et al. 2014, LARDO et al. 2015).

A variante C3435T (rs1045642) foi a primeira a ser descrita por modificar a expressão da proteína P-gp (HOFFMEYER, et al. 2000). Segundo estudos na literatura, pacientes que possuem o genótipo 3435TT têm a especificidade do substrato diminuída e por isso apresentam níveis mais baixos de P-gp no intestino e redução do mRNA do gene *ABCB1* (WANG et al. 2005; KIMCHI-SARFATY et al. 2007; DEENIK et al., 2010; VINE et al., 2014)

Zheng et al. (2015) elaboraram uma meta-análise reunindo 12 estudos que totalizaram 1826 pacientes e relataram associações significativas entre os alelos 2677G (rs2032582) e 3435T (rs1045642) com uma pior resposta ao imatinibe em todas as populações estudadas, enquanto o genótipo 1236CC (rs1128503) previu melhor resposta especificamente em populações asiáticas. O resultado em populações asiáticas foi corroborado em uma meta-análise separada, confirmando a associação entre o locus 1236T> C (rs1128503) com a resposta ao imatinibe neste grupo demográfico (ZU et al., 2014).

O conjunto desses resultados revela que, diretamente ou indiretamente, a expressão desse gene é um fator importante na determinação da concentração intracelular do imatinibe (ANGELINI, et al. 2013; RINALDI *et al.,* 2019). Assim a literatura aponta que o *ABCB1* pode ser utilizado como marcador de resposta precoce em pacientes com LMC que recebem imatinibe (NÉMETHOVÁ; RÁZGA, 2017; EADIE et al., 2016; BEDEWY; ELMAGHRABY; KANDIL, 2019; RAJAMANI *et al.,* 2020).

1. 7. 2. 2. *ABCG2*

O gene *ABCG2* (ATP-binding cassette super-family G member 2), localizado na região 22 do braço longo do cromossomo 4, codifica a proteína BCRP (Breast cancer resistance protein) e está envolvido no tráfego de moléculas dentro da célula através das membranas

celulares (ROBEY et al., 2009). A BCRP é responsável pelo transporte de xenobióticos, incluindo diversos medicamentos e por isso tem sido associado à resistência à tratamentos já que exporta ativamente os compostos das células, principalmente em células cancerígenas (AU et al., 2014).

Como citado anteriormente, a proteína BCRP é uma das proteínas que realiza o transporte do imatinibe e a literatura aponta que alterações na expressão deste gene podem afetar a resposta ao tratamento. Um estudo com 188 paciente com LMC, mostrou que os níveis de expressão do *ABCG2* eram maiores em pacientes com resistência ao imatinibe, assim como em pacientes que não alcançaram a resposta molecular maior (LIMA et al., 2014)

As alterações na expressão do gene podem ser decorrentes de variantes genéticas, como demonstraram Morisaki et al. (2005), que em experimentos in vitro utilizando vários substratos foi verificado que o polimorfismo C421A (rs2231142) do gene *ABCG2*, afeta a eficiência do transporte de BCRP, o que pode resultar em alteração nos perfis de farmacocinética e resistência aos fármacos (MORISAKI, et al. 2005).

Além disso, estudos mostraram que pacientes com LMC que possuíam os genótipos selvagens dos polimorfismos 421CC (rs2231142) e 34GG (rs2231137) tiveram uma menor concentração de imatinibe no plasma e a necessidade de uma dose ajustada com maior concentração do fármaco, enquanto a presença do alelo mutante aumentaram a resposta ao imatinibe e de acordo com Shinohara et al. (2013), este poderia ser um marcador independente para prever resposta molecular nos pacientes(PETAIN, et al., 2008; KIM et al., 2009; TAKAHASHI et al., 2010).

Para reforçar a influência deste gene na resposta ao tratamento com imatinibe, Jiang et al. (2017) realizaram uma meta-análise onde foram avaliados 14 estudos com um total de 2184 pacientes. Obteve-se como conclusão que o genótipo mutante AA do polimorfismo rs2231142 está significativamente associada com maior taxa de resposta molecular e resposta citogenética completas.

1. 7. 2. 3. *SLC22A1*

O gene *SLC22A1((Solute Carrier Family 22 Member 1),* também conhecido como OCT1 (*organic cation transporter 1*), está localizado no cromossomo 6 e tem como função transportar cátions orgânicos, como medicamentos e outros compostos ambientais, do sangue para as células epiteliais (KOEPSELL, 2003). É um dos mais importantes transportadores do fígado localizado na membrana sinusoidal dos hepatócitos e tem afinidades para compostos endógenos e para compostos exógenos como alguns fármacos (JONKER; SCHINKEL, 2004).

Este gene desempenha um papel importante no influxo do imatinibe (BOUCHET et al., 2013; THOMAS et al., 2004; WANG et al, 2008). Assim, a variação dos níveis de expressão deste gene está significativamente associada com biodisponibilidade do fármaco que por sua vez está correlacionada com as respostas citogenéticas e moleculares. Além disso, o gene *SLC22A1* abriga vários polimorfismos que estão associados a uma atividade de transporte alterada (WHITE et al., 2008; KERB et al., 2002; POLLILO et al., 2015; CARGNIN *et al.,* 2018).

Segundo relatos da literatura, pacientes com LMC que tem baixa atividade do gene *SLC22A1* tem menos probabilidade de alcançar a resposta molecular maior (WHITE et al., 2006), enquanto os que tem elevados níveis de expressão mostraram melhores taxas de sobrevivência e maior chance de alcançar respostas citogenética ou molecular (MARIN et al., 2010; WANG et al., 2008; CROSSMAN et al., 2005; CHHIKARA *et al.,* 2017). Condizente com isto, Lima et al (2014) indica que há uma alta expressão do mRNA em pacientes que responderam bem ao tratamento, associando assim que uma alta expressão de *SLC22A1* deve estar associado com sucesso terapêutico em pacientes com LMC (VIVONA et al., 2014).

Alguns estudos avaliaram a relação de variantes genéticas nesse gene com a resposta ao imatinibe: o genótipo c.480CC (rs683369) foi associado com níveis maiores de sobrevida livre de eventos do que os genótipos CG ou GG (DI PAOLO et al., 2014). Já Giannoudis et al. (2013), inferem que pacientes com o alelo M420del do polimorfismo 1260-1262delGAT tem maior possibilidade de não alcançar resposta ótima ao tratamento. E Singh et al. (2012) sugere que polimorfismos e haplótipos específicos nos genes *SLC22A1-ABCB1* podem contribuir na variação de resposta do imatinibe em pacientes com LMC.

1. 7. 2. 4. Outros genes transportadores

Além dos transportadores de efluxo e influxo citados acima, outros transportadores transmembrana podem produzir variabilidade na resposta a ITQs e por isso vão ser investigados no presente trabalho: *ABCC2* (*ATP-binding cassette subfamily A member 2*) e *ABCC4* (*ATP-binding cassette subfamily A member 4)* que também fazem parte da família ABC. E os genes *SLC22A4* (*Solute carrier family 22 member 4*), *SLC22A5* (*Solute carrier family 22 member 4)*, *SLC22A7* (*Solute carrier family 22 member 4*), *SLC29A1* (*Solute Carrier Family 29 Member 1*) e *SLCO1A2* (*Solute carrier organic anion transporter family member 1A2*).

Ressalta-se a importância de estudar transportadores adicionais, pois já foi relatado que quando o imatinibe não se ligou à proteína OCT1 (expressa pelo gene *SLC22A1)* para o influxo houve maior expressão de outros transportadores (HU et al., 2008). Além disso, variantes desses

genes têm sido relacionadas a resposta ao tratamento com imatinibe em pacientes com LMC (YAMAKAWA et al., 2011a; YAMAKAWA et al., 2011b; ANGELINI et al., 2013; GALIMBERTI et al., 2014; LIMA, et al., 2015; JARUSKOVA et al., 2017; OMRAN et al., 2020).

1. 7. 2. 5. Genes metabolizadores

Os genes *CYP3A4* e *CYP3A5* (*Cytochrome P450 Family 3 Subfamily A Member 4 and 5*) fazem parte da família de enzimas Citocromo P450 que realizam a metabolização de fase I de vários compostos xenobióticos e participam ativamente do metabolismo do imatinibe (BOLTON et al, 2004; SAIZ-RODRÍGUEZ *et al,* 2020). Acredita-se que estes genes sejam responsáveis pela expressão da maior parte das enzimas metabolizadoras de CYP no fígado com cerca de 30% e mais de 70% no intestino (DUTREIX et al., 2004; PENA et al., 2020).

O imatinibe é convertido pelas enzimas CYP3A em seu principal metabólito N-desmetil imatinibe, que é farmacologicamente ativo, mas 3–4 vezes menos citotóxico do que o imatinibe (GRÉEN et al., 2010). A importância das enzimas CYP3A foi destacada em um estudo que demonstrou que pacientes com LMC que alcançaram a resposta molecular completa possuíam atividade enzimática de CYP3A4 e CYP3A5 mais alta do que em pacientes com respostas moleculares parciais (MLEJNEK et al., 2011).

A CYP3A4 é a principal enzima responsável pelo metabolismo de primeira passagem dos ITQs, enquanto outras enzimas, incluindo CYP3A5, CYP2C8 e CYP2D6 também estão envolvidas em menor extensão (WHIRL-CARRILLO et al., 2012).

O SNP rs2740574 A>G está situado na região reguladora do gene *CYP3A4*, o que pode alterar a atividade de transcrição e em última análise, influenciar a expressão da enzima. Angelini et al., (2013) descreveu que em pacientes com LMC tratados com imatinibe com o genótipo mutante 9 AA para o polimorfismo rs2740574 tem maior probabilidade de alcançar a RMM do que pacientes com os outros genótipos. Estudos in vitro sugerem que este polimorfismo está associado à uma maior expressão de *CYP3A4* (PENG et al., 2004; GRÉEN et al., 2010).

Entre os polimorfismos do *CYP3A5*, o alelo CYP3A5*3 é o mais importante deles pois apresenta uma alta frequência e um claro papel funcional. O alelo CYP3A5*3 é definido pela substituição A6986G (rs776746), que cria um sítio crítico de splicing e a introdução de um códon de parada prematuro (KUEHL, et al, 2001). Segundo Kuehl et al. (2001), os indivíduos que são homozigotos para este alelo têm níveis reduzidos de CYP3A5 e capacidade metabólica reduzida.

No estudo de Kim et al. (2009), o genótipo 6986AA (rs776746) teve um impacto negativo na expressão da proteína resultando na diminuição da resposta citogenética completa, enquanto Takahashi et al. (2010) não achou uma associação entre esse alelo e o ajuste de dose do imatinibe ou a resposta clínica. Em contraste, Sailaja et al. (2010) encontraram uma frequência maior do genótipo 6986GG em pacientes com LMC associado a uma menor ou pouca resposta hematológica.

Além das variantes nos genes *CYP3A4* e *CYP3A5*, serão investigadas variantes no gene *CYP2A6* que também tem sido relacionadas com a resposta ao tratamento com imatinibe (KASSOGUE et al., 2014; BELOHLAVKOVA et al., 2018).

1. 7. 2. 6. Outros genes

Ademais dos genes citados serão investigados os genes *BCL2L11* (Bcl-2-like 11), *GSTP1*(*Glutathione S-Transferase Pi 1*) e *ULK3* (*Unc-51 Like Kinase 3*) que também têm sido relacionados à resposta ao imatinibe no tratamento da LMC como será referenciado abaixo.

O gene *BCL2L11,* faz parte da família BCL-2 de genes, e está envolvido na ativação da apoptose e morte celular por participar da via da MAPK (KURIBARA et al., 2004). Em relação à sua influência no tratamento da LMC, Augis et al (2013) encontraram que a variante c465C>T (rs724710) deste gene está relacionada com menor taxa de resposta ao imatinibe e que esta alteração leva à uma diminuição da taxa de mRNA. No entanto este não é um ponto de consenso na literatura (XU, et al., 2017).

O gene *GSTP1* é membro da família Glutationas S-transferases (GST) é um gene polimórfico que codifica proteínas variantes ativas e funcionalmente diferentes que auxiliam no metabolismo de xenobióticos, desempenham um papel na suscetibilidade ao câncer e contribuem para resistência em tratamentos oncológicos (SAU et al., 2010). Por isso, este gene vem sendo investigado e relacionado, especialmente a variante rs1695, com a resposta ao imatinibe (DAVIES et al., 2014; MAKHTAR et al., 2017; ROSTAMI et al., 2019; DELMOND et al., 2021; BABA et al., 2021)

E por fim o gene *ULK3*, que expressa uma serina/ treonina proteína quinase com papel de reguladora da sinalização de Sonic hedgehog (SHH) e pode induzir autofagia, seguindo a senescência celular (MALOVERJAN et al., 2010). Para o polimorfismo rs2290573 o genótipo GG é associado com menor resposta ao imatinibe em pacientes com LMC quando comparado com pacientes com os genótipos AA e AG (DRESSMAN et al., 2004).

## 1.8 Ancestralidade

As frequências alélicas de importantes *loci* farmacogenéticos variam entre as populações geográficas e isto resulta em uma variação interétnica de respostas aos tratamentos (RODRIGUES et al., 2019; CARVALHO et al., 2020). A existência destas diferenças pode ser um fator importante na interpretação errônea dos resultados visto que podem existir recomendações para o uso ou para restrição de certos medicamentos de acordo com a população (PERERA et al., 2013; SU et al., 2019; YANG et al., 2021).

Um exemplo importante na observação de tolerância farmacológica entre diferentes populações étnicas está na implementação do esquema S-1, que é utilizado na terapia de câncer de estômago, cólon retal, pulmão, pâncreas, cabeça e pescoço (CHUAH et al., 2011). A dose máxima tolerada de S-1 em pacientes ocidentais é substancialmente menor do que em pacientes japoneses. Essa diferença de tolerância está associada a variantes do gene *CYP2A6,* cuja atividade mostra variabilidade interindividual e interpopulacional considerável ((FUJITA et al., 2006; CHUAH et al., 2011).

A amostra da população investigada neste trabalho é altamente miscigenada entre as principais populações que formaram o Brasil: Europeus, Africanos e Ameríndios. Nos últimos anos, nosso grupo de pesquisa tem investigado e demonstrado que as populações tradicionais da Amazônia, como os Ameríndios, possuem um perfil genético diferenciado revelando que algumas mutações deletérias são mais frequentes nestas populações que em outras populações mundiais e possuem variações genéticas exclusivas (CARVALHO et al., 2020; DOBBIN et al., 2021; FERNANDES et al, 2021; RODRIGUES et al., 2022).

Assim, é importante empregar ferramentas capazes de realizar um controle genômico da ancestralidade entre os indivíduos investigados, quantificando individual e globalmente a proporção de mistura entre as populações ancestrais para corrigir o provável efeito do subestruturamento populacional da amostra. Isto é especialmente importante em indivíduos altamente miscigenados como os que foram investigados no presente trabalho (SANTOS et al., 2010; SUAREZ-KURTZ, 2020).

## 2. JUSTIFICATIVA

Na terapia da Leucemia mieloide crônica com o inibidor de tirosina quinase imatinibe, cerca de 30% dos pacientes apresentam resistência ao tratamento, e esta condição está altamente relacionada às alterações em genes de administração, distribuição, metabolismo e excreção do imatinibe. Desta maneira, investigar variantes implicadas nesse processo pode auxiliar o aumento da eficácia terapêutica aos pacientes resistentes ao imatinibe.

A utilização de marcadores preditivos de tratamento possibilita a escolha mais acertada da terapia para os pacientes. O conhecimento prévio da resposta à terapia permite a eleição do fármaco adequado para cada indivíduo, resultando em alcance mais rápido e efetivo do medicamento para o paciente, melhor qualidade de vida, maior sobrevida e diminuição de custos associados ao tratamento.

Os dados obtidos neste trabalho podem ser úteis para adaptação e implantação de protocolos de tratamento individualizados para instituições localizadas na região Norte do Brasil, melhorando a qualidade e o sucesso da abordagem terapêutica. Cabe ressaltar que este trabalho faz parte de uma iniciativa pioneira na investigação das características genéticas e de ancestralidade em pacientes portadores de LMC na região Norte do Brasil.

# 3. OBJETIVOS

## 3.1 Objetivo geral

O presente trabalho tem como objetivo investigar a associação de biomarcadores moleculares em genes de absorção, distribuição, metabolismo e excreção (ADME) com a resposta ou falha na terapia com imatinibe de pacientes com Leucemia mieloide crônica.

## 3.2 Objetivos específicos

1. Realizar a genotipagem de 30 variantes genéticas que estão distribuídas em 16 genes envolvidos na farmacogenômica do imatinibe (*ABCB1, ABCC2, ABCC4, ABCG2, BCL2L11, CYP2A6, CYP3A4, CYP3A5, GSTP1, SLC22A1, SLC22A4, SLC22A5, SLC22A7, SCLA29A1, SLCO1A2* e *ULK3)* e investigar a associação dos marcadores moleculares selecionados com a resistência secundária no tratamento da LMC.

2. Realizar análise de interação entre as variantes dos genes investigados citados acima, relacionando com os desfechos de resistência secundária no tratamento com imatinibe na LMC.

2. Genotipar 32 SNVs em genes associados com o metabolismo de fármacos e o processo carcinogênico (*ABCC1, ABCC2, ABCC3, AMPD1, ARID5B, ATIC, CCND1, CDKN2A, CEBPE, GGH, IKZF1, ITPA, MTHFD1, MTHFR, MTRR, NALCN, NOS3, PIP4K2A, SHMT1, SLCO1B1, SLCO1B3, TLR4, TNFAIP3* e *TPMT)*, a fim de correlacionar as variantes genéticas com a falha terapêutica ao imatinibe ao longo do tratamento em pacientes com LMC.

3. Aplicar um conjunto Multiplex de 61 marcadores do tipo INDEL (Inserção/Deleção) para realizar o controle genômico de ancestralidade individual dos pacientes investigados.

## 4. ATIVIDADES DE PESQUISA DESENVOLVIDA PELA PROPONENTE DURANTE O DOUTORADO

A primeira contribuição desenvolvida a partir das atividades realizadas durante o período deste doutorado foi a publicação do artigo: **Impact of Variants in the *ATIC* and *ARID5B* Genes on Therapeutic Failure with Imatinib in Patients with Chronic Myeloid Leukemia** (CAPÍTULO I) na revista *Genes* (Fator de impacto: 4.141), abrangendo a relação de variantes envolvidas no metabolismo de fármacos e carcinogênese com a falha na terapia ao imatinibe.

A segunda e principal contribuição desta tese é a publicação do artigo: **Alterations in pharmacogenetic genes and their implications for imatinib resistance in CML patients from an admixed population** (CAPÍTULO II) que está em processo de submissão na revista *Leukemia research*, periódico de grande importância para a comunidade acadêmica (Fator de impacto: 3.715). Este artigo foi desenvolvido em uma cooperação internacional com a Universidad de Santiago de Compostela, no Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS) sob a orientação do prof. Dr. Angel Carracedo e supervisão da Dra. Raquel Cruz Guerrero.

Além disso, foi publicado no periódico *Molecular Genetics and Genomic Medicine* (Fator de impacto: 2.183), o artigo: **Polymorphisms in the *CYP2A6* and *ABCC4* genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population** (ANEXO I) versando sobre a influência de marcadores genéticos na susceptibilidade à LMC.

## CAPÍTULO I: Impact of Variants in the *ATIC* and *ARID5B* Genes on Therapeutic Failure with Imatinib in Patients with Chronic Myeloid Leukemia

Article

# Impact of Variants in the *ATIC* and *ARID5B* Genes on Therapeutic Failure with Imatinib in Patients with Chronic Myeloid Leukemia

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**Abstract:** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm derived from the balanced reciprocal translocation of chromosomes 9 and 22 t (9q34 and 22q11), which leads to the formation of the Philadelphia chromosome and fusion of the *BCR-ABL* genes. The first-line treatment for CML is imatinib, a tyrosine kinase inhibitor that acts on the BCR-ABL protein. However, even though it is a target-specific drug, about 25% of patients do not respond to this treatment. The resistance mechanisms involved in this process have been investigated and studies have shown that germinal alterations can influence this mechanism. The aim of this work was to investigate 32 polymorphisms in 24 genes of carcinogenic pathway to verify the influence of these genetic variants on the response to treatment with imatinib. Our results demonstrated that individuals with the recessive GG genotype for the rs2372536 variant in the *ATIC* gene are approximately three times more likely to experience treatment failure with imatinib ($p = 0.045$, HR = 2.726, 95% CI = 0.9986–7.441), as well as individuals with the TT genotype for the rs10821936 variant in the *ARID5B* gene, who also have a higher risk for treatment failure with imatinib over time ($p = 0.02$, HR = 0.4053, IC 95% = 0.1802–0.911). In conclusion, we show that variants in the *ATIC* and *ARIDB5* gene, never screened in previous studies, could potentially influence the therapeutic response to imatinib in patients treated for CML

**Keywords:** chronic myeloid leukemia; imatinib; *ATIC* gene; *ARIDB5* gene; pharmacogenomics

## 1. Introduction

Chronic myeloid leukemia (CML) has as its main characteristic the reciprocal and balanced translocation of chromosomes 9 and 22 t (9q34 and 22q11), which results in the Philadelphia chromosome and fusion of the *BCR-ABL* genes [1,2]. The expression of this gene is constitutive with tyrosine kinase activity, and it is responsible for leukemogenesis and maintenance of carcinogenic activity in CML [3,4].

Treatment of CML is performed with the tyrosine kinase inhibitor (TKI), imatinib, which acts directly on the BCR-ABL protein by decreasing its intracellular activity and,

thus, controlling the carcinogenic environment [5]. However, even though this is a target-specific drug, the response to this treatment is variable, therefore, about 25% of patients are not responsive to imatinib [4,6].

Variations in response to treatment can be influenced by several factors, including alterations in drug metabolism genes. Genetic variants can alter gene expression and, thus, modulate the interaction of the expressed protein with the drug, making the response inefficient [7–10].

Another factor that can interfere with treatment responses is the genetic composition of a population. It is known that the responses among populations vary worldwide, due to different frequencies of genetic variants in genes involved in absorption, distribution, metabolism, and excretion (ADME) of drugs [11–13]. In addition, in this study, the population investigated is highly mixed, and it is important to carry out a genomic control based on genetic ancestry, therefore, that there is no population substructuring and ancestry is not a confounding factor in the analyses [14,15].

Thus, this study investigated 32 polymorphisms in 24 carcinogenic pathway genes: *ABCC1, ABCC2, ABCC3, AMPD1, ARID5B, ATIC, CCND1, CDKN2A, CEBPE, GGH, IKZF1, ITPA, MTHFD1, MTHFR, MTRR, NALCN, NOS3, PIP4K2A, SHMT1, SLCO1B1, SLCO1B3, TLR4, TNFAIP3*, and *TPMT*, aiming to verify the influence of these genetic variants on the response to treatment with imatinib.

## 2. Materials and Methods

### *2.1. Ethics, Consent, and Permissions*

This study was approved by the Research Ethics Committee of the participating institutions, at the Ophir Loyola Hospital under license number 1.575.920/2016 and at the Núcleo de Pesquisas em Oncologia (NPO) under protocol number 3.354.571/2019. All participants agreed to participate in the research and signed an informed consent form allowing the use of their clinical and genetic data.

### *2.2. Investigated Population*

We investigated a total of 165 patients diagnosed with CML, followed for at least 1 year of treatment at Hospital Ophir Loyola, a reference hospital in the onco-hematology service in the city of Belém do Pará, in Northern Brazil. All patients started treatment with imatinib mesylate and had a detailed clinical follow-up. For the analyses, the patients were divided into two groups: patients who responded well to treatment and patients who did not respond well to treatment. The criteria used to define the hematologic and molecular response followed the National Comprehensive Cancer Network [16].

### *2.3. Selected Markers*

For marker selection, the criteria were based on PharmGKB, NCBI, and Ensembl databases, as well as data available in literature regarding important variables for the carcinogenic pathway. The description of markers can be found in the Supplementary Materials Table S1.

### *2.4. DNA Extraction and Quantification*

Genetic material was extracted from peripheral blood collected in EDTA tubes and using an Axy PrepTM Blood Genomic DNA Miniprep kit (Axygen Biotechnology, San Francisco, CA, USA), following the manufacturer’s instructions. The DNA concentration and purity were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000, NanoDrop Technologies, Wilmington, DE, USA).

### *2.5. Genotyping*

Genotyping of the samples was performed on a QuantStudio™ 12K Flex Real-Time PCR system (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using real-time

PCR technology (TaqMan OpenArray Genotyping) by allelic discrimination, following all the manufacturer's recommendations.

### 2.6. Genetic Ancestry

An ancestry analysis was performed as described by Ramos et al. [17] using 61 autosomal ancestry informative markers (AIMs) in three multiplex PCR reactions, aiming to accurately estimate the individual and global interethnic mix [14]. Amplicons were analyzed using an ABI Prism 3130 sequencer (Thermo Fisher Scientific, Waltham, MA, USA) and Gene Mapper ID v.3.2 software (Thermo Fisher Scientific, Waltham, MA, USA). The proportions of individual genetic ancestors were estimated using the STRUCTURE v.2.3.3 software (Stanford University, CA, USA), assuming three parental populations. This analysis was performed to control a possible population substructure, as the investigated population was highly mixed.

### 2.7. Statistical Analysis

To be included in the statistical analyses, the genotyping data needed at least 70% in coverage. The allelic and genotypic distribution is shown in Supplementary Table S2.

The statistical analyses were run in SNPassoc library in RStudio v.3.6.1 software (Boston, MA, USA). Differences in the categorical variable (sex) were tested using Pearson's chi square, while the quantitative variable (mean age) was evaluated using Student's t-test. The ancestry indices were compared between the groups using the Mann–Whitney test. Multiple logistic regressions were used to assess possible associations between the polymorphisms and the response to treatment with imatinib, by estimating the odds ratios (ORs) and their 95% confidence intervals (CIs). The Kaplan–Meier survival analysis was used to estimate possible differences in the time of loss of response for each genotype by estimating the hazard ratio (HR). For this statistical test, we evaluated the variable "time of treatment failure" (TTF)—an event in which treatment was changed, due to absence of molecular or cytogenetic responses or intolerance to treatment. A significance level of $p < 0.05$ was considered for all the statistical analyses.

## 3. Results

The clinical epidemiological data are shown in Table 1, showing that, among the 165 patients included in this study, 103 (63.1%) patients had an excellent response to treatment and 62 (36.9%) patients did not. When comparing the variable "age at diagnosis" between the groups (responders and non-responders) we found no significant difference (*p*-value = 0.451), the same result was found when gender distribution was analyzed (*p*-value = 0.078). Regarding the genetic ancestry in both groups, we also found no significant difference between them.

**Table 1.** Clinical and epidemiological variables of the investigated patients.

| Variable | Responders 103 (63.1%) | No Responders 62 (36.9%) | *p*-Value |
|---|---|---|---|
| **Age (years)** | 48.50 ± 15.07 | 46.76 ± 15.00 | 0.451 |
| **Sex (%)** | | | 0.078 |
| Men | 64 (62.1) | 29 (46.7) | |
| Women | 39 (37.9) | 33 (53.3) | |
| **Ancestry Mean** | | | |
| European | 0.468 ± 0.147 | 0.458 ± 0.147 | 0.797 |
| Amerindian | 0.299 ± 0.132 | 0.319 ± 0.133 | 0.604 |
| African | 0.233 ± 0.101 | 0.223 ± 0.101 | 0.686 |

Further information on clinical data reveals that 34 (20.2%) patients failed to respond during treatment (mean 47.11 months) and 12 (7.14%) patients were unresponsive from the beginning of treatment.

### 3.1. Genotype and Imatinib Response-Relative Risk Assessment (OR)

Regarding genetic variants, we found no association between responsiveness to therapy at a specific time and the investigated SNPs (Supplementary Table S3).

### 3.2. Time of Treatment Failure (TTF)/Risk Analysis over the Response Time (HR)

We investigated the relationship between genetic variants and the variable "time of treatment failure" (TTF) to estimate the risk of treatment failure over time. Our results indicate that the SNVs rs2372536 in the *ATIC* gene and the rs10821936 in the *ARID5B* gene were statistically significant (Table 2).

**Table 2.** Hazard ratio analysis of the genotypes analyzed with the time of treatment failure.

| Genotype | HR (95% CI) | Lower | Upper | *p*-Value |
|---|---|---|---|---|
| *ATIC* rs2372536 | | | | |
| CC/CG vs. GG [1] | 2.726 | 0.9986 | 7.441 | 0.04 |
| CC vs. CG + GG [2] | 1.484 | 0.7235 | 3.046 | 0.3 |
| *ARID5B* rs10821936 | | | | |
| TT vs. CT + CC [1] | 0.4053 | 0.1802 | 0.911 | 0.02 |
| CC vs. TT + CT [2] | 0.6114 | 0.2436 | 1.535 | 0.3 |
| *TPMT* rs1142345 | | | | |
| TT + CT vs. CC [1] | 0.711 | 0.165 | 3.063 | 0.6 |
| TT vs. CT + CC [2] | 0.9895 | 0.4341 | 2.255 | 1.00 |
| *TPMT* rs12201199 | | | | |
| NA [1,3] | | | | |
| AA vs. AT + TT [2] | 0.6824 | 0.2561 | 1.818 | 0.4 |
| *SLC01B1* rs4149056 | | | | |
| NA[1,3] | | | | |
| TT vs. CT + CC [2] | 1.109 | 0.3502 | 2.323 | 0.8 |
| *ABCC2* rs717620 | | | | |
| NA [1,3] | | | | |
| CC vs. CT + TT [2] | 1.909 | 0.1807 | 1.518 | 0.2 |
| *ABCC3* rs9895420 | | | | |
| NA [1,3] | | | | |
| TT vs. AT + AA [2] | 1.738 | 0.1983 | 1.67 | 0.3 |
| *GGH* rs11545078 | | | | |
| NA [1,3] | | | | |
| GG vs. AG + AA [2] | 1.769 | 0.81 | 3.862 | 0.1 |

| | | | | |
|---|---|---|---|---|
| *GGH* rs3758149 | | | | |
| GG + AG vs. AA [1] | 0.9296 | 0.2162 | 3.997 | 0.9 |
| GG vs. AG + AA [2] | 1.148 | 0.5384 | 2.446 | 0.7 |
| *ATIC* rs4673993 | | | | |
| TT + CT vs. CC [1] | 2.561 | 0.3905 | 0.9388 | 0.06 |
| TT vs. CT + CC [2] | 1.633 | 0.7888 | 3.382 | 0.2 |
| *AMPD1* rs17602729 | | | | |
| NA [1,3] | | | | |
| GG vs. AG + AA [2] | 0.7357 | 0.2756 | 1.964 | 0.5 |
| *CCND1* rs9344 | | | | |
| GG + AG vs AA [1] | 1.536 | 0.5155 | 4.576 | 0.4 |
| GG vs. AG + AA [2] | 1.174 | 0.5439 | 2.532 | 0.7 |
| *IKZF1* rs4132601 | | | | |
| TT + GT vs. GG [1] | 0.7569 | 0.1013 | 5.657 | 0.8 |
| TT vs. GT + GG [2] | 1.164 | 0.5258 | 2.579 | 0.7 |
| *ITPA* rs1127354 | | | | |
| CC vs. AC [1] | 1.957 | 0.4465 | 8.575 | 0.4 |
| *MTRR* rs1801394 | | | | |
| AA + AG vs. GG [1] | 0.8794 | 0.328 | 2.358 | 0.8 |
| AA vs. AG + GG [2] | 0.9615 | 0.4536 | 2.038 | 0.9 |
| *MTHFD1* rs2236225 | | | | |
| GG + AG vs. AA [1] | 0.6535 | 0.2462 | 1.735 | 0.4 |
| GG vs. AG + AA [2] | 0.7404 | 0.3318 | 1.652 | 0.5 |
| *NOS3* rs1799983 | | | | |
| NA [1,3] | | | | |
| GG vs. GT [2] | 1.052 | 0.2408 | 4.592 | 0.9 |
| *MTHFR* rs1801133 | | | | |
| GG + GA vs. AA [1] | 1.445 | 0.4198 | 4.97 | 0.6 |
| GG vs. GA + AA [2] | 0.9837 | 0.4521 | 2.141 | 1.00 |
| *TLR4* rs4986790 | | | | |
| NA [1,3] | | | | |
| AA vs. AG [2] | 1.545 | 0.6471 | 0.2034 | 0.7 |
| *TPMT* rs1800460 | | | | |
| CC vs. CT + TT [1] | 0.6339 | 0.218 | 1.843 | 0.4 |
| NA[2] | | | | |

| | | | | |
|---|---|---|---|---|
| *SLCO1B1* rs4149015 | | | | |
| GG vs. AG + AA [1] | 0.8043 | 0.2271 | 2.849 | 0.7 |
| AA vs. GG + AG[2] | 3.472 | 0.288 | 0.4051 | 0.2 |
| *GGH* rs1800909 | | | | |
| AA+AG vs. GG [1] | 0.8698 | 0.2995 | 2.526 | 0.8 |
| NA [2] | | | | |
| *NALCN* rs7992226 | | | | |
| AA+AG vs. GG [1] | 0.03471 | 0.42 | 2.552 | 0.9 |
| AA vs. AG + GG [2] | 1.199 | 0.348 | 4.132 | 0.8 |
| *SHMT1* rs1979277 | | | | |
| AA+AG vs. GG [1] | 0.873 | 0.3515 | 2.168 | 0.8 |
| GG vs. AA + AG [2] | 3.361 | 0.6504 | 1.447 | 0.1 |
| *SLCO1B1* rs2306283 | | | | |
| GG+AG vs. AA [1] | 1.733 | 0.6368 | 4.718 | 0.3 |
| GG vs. AG + AA [2] | 1.276 | 0.7837 | 0.4663 | |
| *CEBPE* rs2239633 | | | | |
| GG+AG vs. AA [1] | 1.899 | 0.8027 | 4.493 | 0.1 |
| GG vs. AG + AA [2] | 2.229 | 0.4485 | 0.506 | 0.3 |
| *TNFAIP3* rs6920220 | | | | |
| GG+AG vs. AA [1] | 0.6463 | 0.2426 | 1.722 | 0.4 |
| NA [2] | | | | |
| *PIP4K2A* rs7088318 | | | | |
| AA vs. AC + CC [1] | 0.5205 | 1.921 | 0.1135 | 0.4 |
| NA [2] | | | | |

[1] Recessive model; [2] Dominant model; [3] Not applicable for analysis.

According to our results, we can infer that individuals with the recessive GG genotype for the rs2372536 variant in the *ATIC* gene are approximately three times more likely to experience treatment failure with imatinib ($p$ = 0.045, HR = 2.726, 95% CI 0.9986–7.441), as well as individuals with the TT genotype for the rs10821936 variant in the *ARID5B* gene, who also have a higher risk for treatment failure with imatinib over time ($p$ = 0.02, HR = 0.4053, IC 95% 0.1802–0.911) (Figure 1).

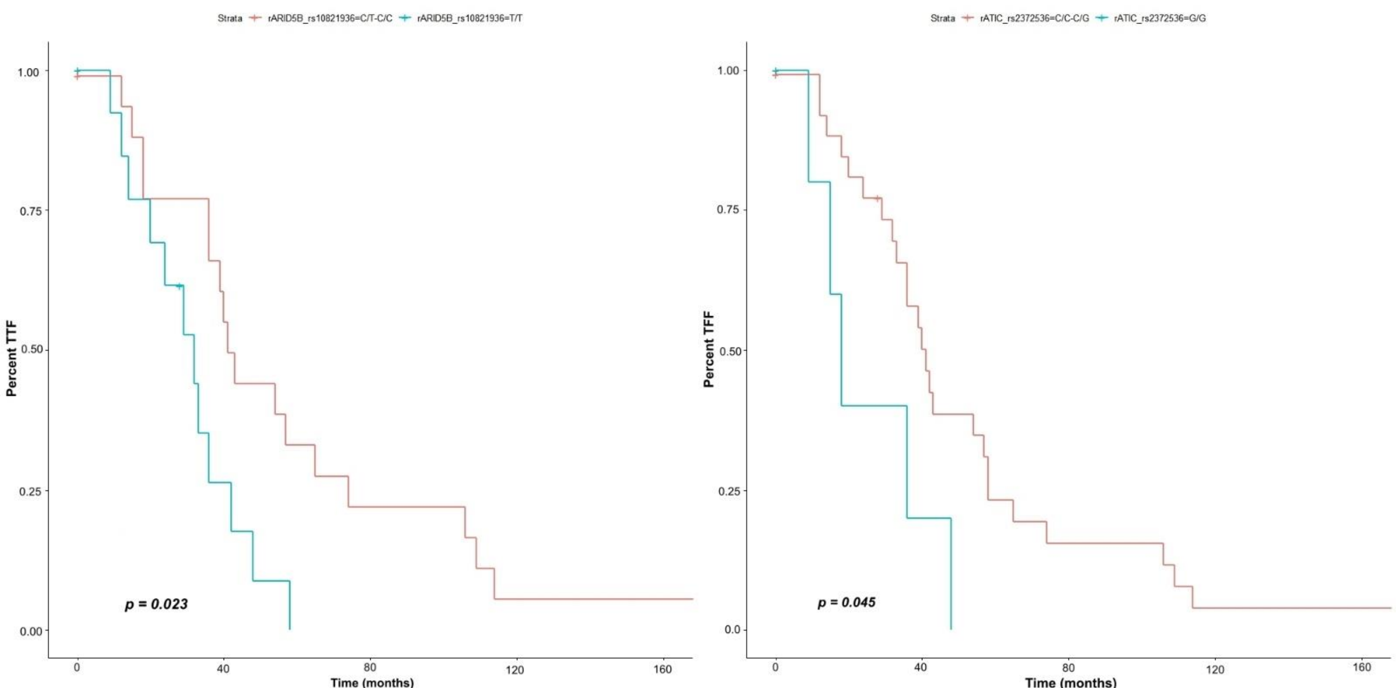


**Figure 1.** Kaplan–Meier curve demonstrating time of treatment failure (TTF) associated with rs10821936 of *ARID5B* gene and rs2372536 variant of *ATIC*.

## 4. Discussion

Imatinib (STI-571is a 2-phenylamino-pyrimidine compound that inhibits the autophosphorylation of the BCR-ABL protein. This happens through the binding of TKI to the ATP receptor (adenosine triphosphate), which does not allow the binding of the phosphate group of the ATP molecule, keeping the protein inactivated. In this way, the entire downstream signaling cascade is turned off and the leukemic cells stop dividing [4,5].

This drug is used primarily for the treatment of CML, gastrointestinal stromal tumors (GISTs), and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL Ph+) [18]. Imatinib was the first TKI that showed efficiency in the treatment of CML, and it was approved by the Food and Drug Administration (FDA) of United States of America and also by Agência Nacional de Vigilância Sanitária (Anvisa of Brazil) in 2001, soon beginning to be used as a first-line treatment for CML.

In addition to inhibiting the BCR-ABL protein, imatinib also works as an inhibitor of other signaling pathways, such as those activated by the platelet-derived growth factor receptor (PDGFR), c-Kit (type III member of kinase receptors), MAPK (mitogen-activated protein kinase), and PI3K/AKT (phosphatidyl inositol 3 kinase), thus, acting in several ways to block cell division [19].

Resistance to imatinib therapy occurs in about 25% of patients with chronic myeloid leukemia; the mechanisms involved in this process have been investigated and studies show that genetic and epigenetic alterations can influence it [7].

In this study, genetic variants in genes involved in the carcinogenic pathway drugs were investigated in order to understand their influence on the response to treatment with imatinib in patients with CML. Our results demonstrated a significant association between the rs2372536 of the *ATIC* gene and rs10821936 of *ARID5B* gene treatment failure with imatinib.

### *4.1. ATIC*

The *ATIC* gene (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) it is located on chromosome 2q35 and encodes a bifunctional enzyme that catalyzes the last two steps of purine biosynthesis, generating inosine

monophosphate from the aminoimidazole carboxamide ribonucleotide. Its pathways are related to AMP-activated protein kinase signaling (AMPK) and to antifolate resistance, as it is able to convert (5-amino-1-(5-phospho-β-D-ribosyl) imidazole-4-carboxamide)) into 6-mercaptopurine ribonucleotide, an inhibitor of purine biosynthesis used in the treatment of leukemias [20].

The de novo purine synthesis pathway is important for the disordered tumor growth process, as it is part of an anabolic pathway of cell multiplication; it is widely used in metabolic reprogramming for cell survival [21,22]. However, the specific role of the *ATIC* gene in modulating cancer progression remains unknown [23–25].

This gene has been shown to be overexpressed in hepatocellular carcinoma (HCC ) and related to a worse prognosis in this neoplasm. The authors found that ATIC activates mTOR-S6 kinase 1 signaling and consequently stimulates the proliferation and migration of oncotic cells [25]. Furthermore, the gene has been associated with autophagy and an increased risk of developing HCC [26], lung cancer [27], and multiple myeloma [28].

In addition, the *ATIC* gene is associated with the risk of lymphoma progression in cases of ATIC protein fusion with the protein of oncogene *ALK* (anaplastic lymphoma kinase) [29,30]. This fusion even influences the treatment because when ALK phosphorylates ATIC in Y104, there is an increase in enzymatic activity. ALK-mediated phosphorylation of ATIC can rescue cancer cells from cell death induced by antifolate agents [31]. These results together suggest that *ATIC* may play an important role in carcinogenesis and cancer cell survival even under treatment [32].

This type of relationship of *ATIC* gene variants has also been demonstrated in other investigations with cancer therapeutic resistance such as in the treatment of breast cancer with tamoxifen [33], the use of pemetrexed for non-small cell lung cancer [34], as well as the use of methotrexate for rheumatoid arthritis [35], pediatric osteosarcoma [36], and acute lymphoblastic leukemia [37]. The investigated rs2372536 polymorphism is a missense mutation, responsible for the substitution of a threonine for a serine at position 116 of exon 5 of the expressed protein (c.347C > G; Thr116Ser), and it is one of the main biomarkers investigated in the response to methotrexate in rheumatoid arthritis [38–40].

### *4.2. ARID5B*

The *ARID5B* gene is part of the AT-rich interaction domain (ARID) family of DNA-binding proteins, which are described as chromatin remodeling factors and also responsible for regulating the transcription of target genes [41,42]. ARID5B forms a complex with the PHF2 protein, which has H3K9me2 histone demethylase activity. H3K9me2 is one of the main markers of silenced chromatin and, thus, there is an epigenetic regulation of gene expression [43].

In addition, recent findings demonstrate that ARID5B is involved in cell proliferation and acts in the growth and differentiation of progenitor B-lymphocytes. It is a co-activator that binds to the 5′-AATA(CT)-3′ sequence [43]. The rs10821936 is a variant located in intron 3, and variants present in this intron are the most associated with susceptibility to ALL [44–47]. Although its role in leukemogenesis is not fully understood, SNPs in intron 3 of *ARID5B* may alter the transcription network involving normal hematopoiesis, thus, altering cell growth and differentiation [48].

Variants in the *ARID5B* gene have also been related to ALL regarding relapse and treatment response [49]. It has also been associated with risk of developing colorectal cancer [50], participation in breast cancer metabolism [51], and with a protein with unregulated function in prostate cancer [52]. These findings suggest that this gene plays an important role in the carcinogenic process.

Therefore, we suggest that variants of the *ATIC* and *ARID5B* genes may interfere with imatinib response in patients with CML, once, even though these genes do not interact directly with the drug, they act in the cellular environment supporting the survival of cancer cells, thus, impairing the effect of the treatment.

## 5. Conclusions

We conclude that the never-before-screened genetic variants of the genes *ATIC* (rs2372536) and *ARID5B* (rs10821936) play a role in therapeutic failure with imatinib, the gold standard treatment for CML.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Polymorphisms chosen after applying the criteria of selection, Table S2. Allelic and genotypic distribution and quality control of the polymorphisms, Table S3. Odds ratio and genotype distributions of the polymorphisms not statistically significant between the patients with response and without response.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research Ethics Committees of the Núcleo de Pesquisas em Oncologia, under protocol number 3.354.571/2019, and by the Hospital Ophir Loyola, under protocol number 1.575.920/2016.

**Informed Consent Statement:** All participants agreed to participate in the research and signed an informed consent.

**Data Availability Statement:** The data presently in this study are available on request from the corresponding author. The data are not publicly available due to the privacy topics contained in informed consent.

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**Conflicts of Interest:** The authors declare that they have no competing interests.

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# CAPÍTULO II: Alterations in pharmacogenetic genes and their implications for imatinib resistance in CML patients from an admixed population

# Alterations in pharmacogenetic genes and their implications for imatinib resistance in CML patients from an admixed population

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## ABSTRACT

Imatinib is the tyrosine kinase inhibitor used as the gold standard for the treatment of Chronic Myeloid Leukemia. However, about 30% of patients do not respond well to this therapy. Variants in drug administration, distribution, metabolism and excretion (ADME) genes play an important role in the process of drug resistance. Thus, the aim of this work was to investigate the association of 30 SNVs of genes involved in the ADME of imatinib with to CML response treatment. Our results indicated that for the rs2290573 of the *ULK3* gene, patients with the recessive AA genotype are three times more likely to develop resistance over time (secondary resistance) ($p = 0.019$, OR = 3.19, IC 95%= 1.21-8.36). Finally, we performed interaction analysis between the investigated variants and found several associations between SNVs for secondary resistance. We conclude that the variant rs2290573 of the *ULK3* gene may be relevant for predicting response to treatment of CML with imatinib and possible treatment resistance. The use of predictive biomarkers is an important tool for therapeutic choice of patients, aiding quality of life and treatment efficacy.

## INTRODUCTION

The use of tyrosine kinase inhibitors (TKI) has begun a new era in Chronic Myeloid Leukemia (CML) therapy as it increased patients' life expectancy and quality of life [1]. Imatinib mesylate was the first used TKI and to this day it remains as first choice drug for initiating CML therapy [2, 3].

CML develops from the reciprocal and balanced translocation of chromosomes 9 and 22, resulting in the formation of the Philadelphia chromosome and the *BCR-ABL* gene [4,5].

This is a constitutive tyrosine-kinase activity gene that will trigger and sustain the entire leukemogenic process of CML [6, 7, 8]. Thus, imatinib acts by inhibiting cell proliferation through inactivation of the BCR-ABL protein and its interaction with other accessory cell division pathways [8, 9].

However, even though treatment with imatinib is target-specific, about 30% of patients are not good responders. The resistance mechanisms present in this process have been investigated and are currently divided between BCR-ABL-dependent and BCR-ABL-independent mechanisms [8, 10].

Even though BCR-ABL-dependent mutations are very relevant, they cannot explain 30-50% of imatinib resistance cases [8]. Thus, we highlight the importance of non-BCR-ABL-dependent resistance mechanisms, which are mainly due to alterations in genes involved in drug Absorption, Distribution, Metabolism and Excretion (ADME). Genetic variants can influence the activity of ADME proteins, which modulate the drugs' pharmacodynamics and pharmacokinetics and also the patient's response to treatment [11, 12, 13].

Furthermore, the resistance mechanisms to imatinib can also be divided into primary resistance – when there is no hematologic or molecular response since the start of therapy –, and secondary resistance – when there is an initial response that disappears over the course of treatment [14].

In this study, we focused on the mechanism of BCR-ABL-independent resistance and investigated genetic variants in imatinib transport and metabolism genes associated with treatment resistance.

We emphasize that the population investigated in this research is highly admixed and therefore has singularities in its genomic background. Thus, genomic ancestry was also analyzed in this work in order to avoid genetic understructuring [15, 16] and misinterpretation of the data [17, 18].

This work aims to investigate the association of 30 genetic variants in 16 genes, namely: *ABCB1, ABCC2, ABCC4, ABCG2, BCL2L11, CYP2A6, CYP3A4, CYP3A5, GSTP1, SLC22A1, SLC22A4, SLC22A5, SLC22A7, SLC29A1, SLCO1A2* and *ULK3*, which are involved in the transport and metabolism of imatinib, to study treatment response with this TKI in patients with CML.

## METHODS

### Ethical Aspects

The development of the study was approved by the research ethics committee of the Núcleo de Pesquisa em Oncologia (protocol number 3.354.571/2019) and by the Ophir Loyola Hospital (protocol number 1.575.920/2016). All research participants signed the Informed Consent Form.

### Sample studied

We investigated 129 patients diagnosed with Chronic Myeloid Leukemia, seen at the Oncohematology sector of the Ophir Loyola Hospital (Belém - PA, Brazil), treated with imatinib as first choice for CML therapy, with detailed follow-up of their clinical and epidemiological data. In addition, it is important to emphasize that the participants are highly admixed with a distinct genetic profile, a relevant factor for our study.

We evaluated the treatment response following the international scale criteria [19], which is based on the hematological response – from blood count tests – and the molecular response – from BCR-ABL gene PCR tests. Patients were classified focusing on two types of response to imatinib: (1) primary resistance - patients who showed resistance from the start of treatment and (2) secondary resistance - patients who previously had a good response but stopped responding over time.

After separating the groups, we noticed that the count of patients with primary resistance was small, so we chose to carry out the analyzes only with patients who presented secondary resistance. Thus, were compared these patients with the group of patients who had a good response to imatinib in the treatment of CML.

### Marker Selection

Forty variants in 16 genes of the imatinib ADME pathway were selected and obtained by querying the following databases: "1000 Genomes Project", dbSNP from the National Center for Biotechnology Information (NCBI), PharmGKB and other specialized articles in literature. The description of the 30 selected variants is in Table 1 of supplementary material. To ensure that the results obtained are unrelated to variants in *ABL1* gene – which are widely related to imatinib resistance –, eight variants of this gene were also analyzed as a control method (Table 2 of the supplementary material).

**DNA extraction and quantification**

Genetic material was extracted from peripheral blood of the participants using the commercial DNA extraction kit BiopurKit Mini Spin Plus - 250 (Biopur, Brazil), according to the manufacturer's recommendations. It was quantified with the NanoDrop 1000 spectrophotometer equipment (Thermo Scientific NanoDrop 1000; NanoDrop Technologies, Wilmington, DE).

**Variant genotyping**

Variants investigated were genotyped using the Agena Bioscience™ iPlex Assay technology, which uses the Mass ARRAY technique to identify the DNA sequence. After amplification of the DNA of interest by the polymerase chain reaction (PCR), the iPlex reaction is performed where a single base extension (SBE) with modified molecular mass is added where each nucleotide has a specific weight [20].

Subsequently, the DNA molecules are ionized in a vacuum chamber and accelerated toward a highly sensitive detector (Matrix-Assisted Laser Desorption Ionization - Time of Flight - MALDI-TOF). The speed of molecules is proportional to the mass of the individual extension products that have been added previously (SBE), and separation occurs by time of flight. The software processes the time of flight and differentiates the variants by their mass, producing a mass spectrum, which eliminates the need for fluorescence or any other labeling [21].

**Genomic Ancestry Analysis**

Ancestry analysis was performed as described by Ramos et al. [22] using 61 autosomal ancestry informative markers (AIMs) in order to accurately estimate interethnic admixture (individual and global) in mixed populations with different ethnic groups as described by SANTOS et al. [15]. Three multiplex PCR reactions are performed, and amplicons are analyzed using the ABI Prism 3130 sequencer (Thermo Fisher Scientific, Waltham, MA, USA) and subsequently Gene Mapper ID v.3.2 software (Thermo Fisher Scientific, Waltham, MA, USA). The proportions of individual ancestry are estimated using the STRUCTURE v.2.3.3 software (Stanford University, CA, USA), assuming the three parental populations who make up the Brazilian population primarily - Europeans, Amerindians, and Africans. This analysis was performed in order to control for possible population substructuring due to the high miscegenation of the investigated population.

### Statistical Analysis

Statistical analyses were performed in Software R v.4.0.5 (The R Foundation, 2016), where Pearson's chi-square test was used to analyze categorical variables and Student's t test was used for continuous variables between the groups investigated. As for comparing the levels of ancestry between samples, the Mann-Whitney test was performed. Multiple logistic regression was used to evaluate the association between the selected genetic variants and the clinical-epidemiological characteristics, estimate the odds ratio (OR) and the 95% confidence interval (CI). Afterwards, interaction analyses were performed between the markers evaluated, based on the recessive model of the genotypes and the secondary resistance as the dependent variable of the analysis. All statistical tests were performed using the SNPassoc library of R, based on a two-tailed probability and a p value ≤ 0.05 was considered significant.

## RESULTS

**Table 1.** Epidemiological data of patients investigated.

| **Variable** | **Responders (%)** **77 (74.03)** | **Secondary resistance (%)** **27 (25.96)** | ***p*-Value** |
|---|---|---|---|
| **Age (years)** | 48.02(±16.19) | 42.59 (±15.96) | 0.131 |
| **Sex (%)** | | | 0.125 |
| Women | 31 (40.3) | 7 (25.9) | |
| Men | 46 (59.7) | 20 (74.1) | |
| **Ancestry Mean** | | | |
| European | 0.494(±0.149) | 0.493(±0.151) | 0.976 |
| Amerindian | 0.274(±0.132) | 0.324((±0.131) | 0.132 |
| African | 0.231(±0.098) | 0.182(±0.099) | 0.073 |

Analysis of the clinical and epidemiological data revealed that, among the 129 patients investigated, 77 (74.03%) were good responders to treatment and 27 (25.96%) presented secondary resistance to imatinib. Epidemiological variables were compared between groups, however, there were no significant differences in any variable analyzed (age at diagnosis, gender, and genetic ancestry).

Subsequently, we analyzed the association between the 30 variants with the patients' clinical response. According to the analyses, all genetic markers analyzed are in Hardy-Weinberg Equilibrium (Available in supplemental Table 3). Results of SNVs analyzes which were not statistically significant are shown in Supplemental Table 4 and 5.

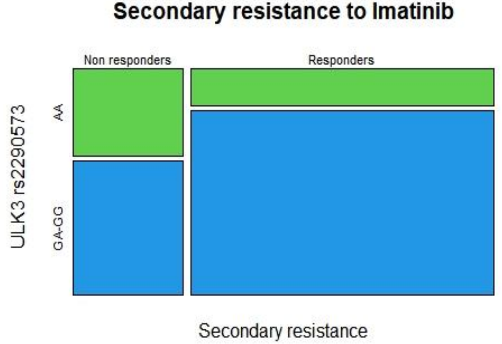
In the analysis of isolated variants, among patients who developed resistance over the course of treatment, a statistically significant result was obtained for the rs2290573 variant of the *ULK3* gene. Thus, patients with the recessive AA genotype are three times more likely to develop resistance over time than patients with other genotypes (Table 2 and figure 1).

**Table 2.** Association between rs2290573 of the *ULK3* gene and secondary resistance to imatinib.

| Genotype | Responders (%) | Secondary resistance (%) | OR (95% CI) | Lower | Upper | p-value |
|---|---|---|---|---|---|---|
| ***ULK3* rs2290573** | | | | | | |
| GG+GA | 64 (83.1) | 17 (60.7) | 1.00 | | | |
| AA [1] | 13 (16.9) | 11 (39.3) | 3.19 | 1.21 | 8.36 | 0.019 |

[1]Recessive model.

**Figure 1.** Distribution of secondary resistance group genotypes vs. other patients.




And when we performed the interaction analysis of the polymorphisms, the results revealed nine statistically significant interactions in which the genes *ABCB1, BCL2L11, GSTP1, SLC29A1, SLCO1A2, SLC22A4, ABCG2, ULK3* and *CYP2A6* are involved. The results are shown in Table 3 and the interactions can be visualized in figure 2, where the regions with the strongest staining are the ones with the strongest interaction.

**Table 3.** Interaction of investigated SNVs in association with secondary resistance to imatinib

| Interaction* | *ABCB1* rs1128503 | *GSTP1* rs1695 | *BCL2L11* rs724710 | *SLC29A1* rs747199 | *SLCO1A2* rs4148977 | *SLCO1A2* rs4148978 |
|---|---|---|---|---|---|---|
| ***ABCB1* rs1045642** | 0.0211 | | 0.010 | | | |
| ***SLC22A4* rs1050152** | | 0.045 | | | | |
| ***ABCB1* rs1128503** | | | 0.038 | | | |
| ***ABCG2* rs12505410** | | | | 0.035 | | |
| ***ULK3* rs2290573** | | | | | 0.043 | 0.043 |
| ***ABCG2* rs2725252** | | | | 0.019 | | |
| ***CYP2A6* rs28399433** | | | 0.007 | | | |

* Recessive model

**Figure 2.** Interaction analysis of variants in patients who showed secondary resistance

# DISCUSSION

Imatinib mesylate is the standard drug for CML treatment, once it is specifically targeted and acts directly on cells with increased tyrosine-kinase activity, leading to leukemic cell death [3, 19]. Despite this, about 30% of patients are not good responders to this treatment [8]. One of the causes associated with this resistance are genetic variants in ADME genes that may interfere and modulate the response to treatment [14,23].

Thus, we investigated the possible association between selected genetic variants with secondary resistance in patients with CML treated with imatinib. In the individual association of the variants, the results revealed a statistically significant result to *ULK3* gene and while evaluating the sum effect of the investigated variants, our work showed interactions between several loci in the candidate genes with imatinib resistance.

## *ULK3*

While performing the analysis of SNVs separately, the first result shows that there was a significant difference for higher risk of resistance associated with the recessive AA genotype of the rs2290573 variant in the *ULK3* gene.

The *ULK3* (Unc-51 Like Kinase 3) gene expresses a protein which has serine/threonine kinase activity, and its functions includes to autophagy, autophosphorylation, and mitotic spindle division of daughter cells during mitosis. ULK3 has been listed as a high potential gene for predicting prognosis in colorectal cancer [23, 24].

The rs2290573 polymorphism was related to imatinib in CML patients in the article by Dressman et al [26]. In this research, it was shown that individuals with the GG genotype have an average of 48% response failure, while individuals with the GA or AA genotypes have an average of 11%, suggesting a better response than the previous group. These findings differ from the results we found, since in our research the AA genotype is more prone to treatment resistance. *ULK3* gene is also involved in the cell cycle and, for this reason, it could influence the resistance of leukemic cells, as seen in another study with CML [27].

## Interaction between polymorphisms

We have more results quantitatively in the interactions analysis in comparison to the isolated variants analysis, which we associate to the probable effect of the mutations addition. The synergistic analysis is a more accurate assessment of the treatment response, since this type of assessment requires a broader view of the context, as is the case in the natural drug pathway [28, 29].

The presence of more than one genetic alteration will lead to a greater loss of metabolic activity of proteins in the microcellular environment, which individually could be minor, however the sum of these effects makes them significant, affecting more persistently the response to treatment [28,29].

The emergence of secondary resistance may also be associated with metabolic loss due to natural aging [30]. Thus, in this scenario we have the presence of two events: the somatic mutations inherent to the individual plus the natural wear and tear accumulated over the years, which contributes to the various associations found.

Variant interaction analysis for patients with secondary resistance to treatment showed significance for nine interactions, among genes *ABCB1, BCL2L11, GSTP1, SLC29A1, SLCO1A2, SLC22A4, ABCG2, ULK3* and *CYP2A6.*

***ABCB1***

The *ABCB1* gene (MDR1, Multi-Drug Resistance Gene 1) encodes the P-glycoprotein (P-gp) protein, whose overexpression has often been linked to chemotherapeutic drug resistance due to its ability to efflux cytotoxic drugs [ 31, 32, 33, 34, 35]. Galimberti et al. [36] evidenced that imatinib is a substrate of P-gp and that *ABCB1* gene expression influences the efficiency of the drug in the treatment of CML.

Zheng et al. [37] conducted a meta-analysis within 12 studies, in which the presence of rs1045642 variant results in significant associations between the homozygous mutant genotype and worse responses to imatinib in all studied populations. The same work showed that the CC genotype of rs1128503 predicted better response to imatinib [37], specifically in Asian populations, which was confirmed by Zu et al. [38].

The interaction found in our work to *ABCB1* gene occurred between two variants of the gene itself (rs1128503 and rs1045642), which is described as haplotype by some study and has been related to pharmacogenetics studies [39, 40, 41], including with CML [29].

Therefore, literature points out that *ABCB1* can be used as an early response marker in CML patients receiving imatinib, since the expression of this gene is an important factor in determining the intracellular concentration of imatinib [28, 42, 43, 44, 45, 46]. Furthermore, both variants reported here have already been associated in other interactions with transporter genes related to treatment response, confirming the results found in this work [47, 48].

***GSTP1* and *SLC22A4***

Another interaction with a significant result was between the *GSTP1* and *SLC22A4* genes. Protein GSTP1 is involved in the susceptibility to some types of cancer, and it contributes to resistance in cancer treatments [49, 50, 51, 52]. The polymorphic variant rs1695 (Ala105Val A>G) leads to low enzymatic activity in the protein [53, 54], and it has been linked to imatinib response in CML treatment [29, 55, 56, 57].

Several studies point out that the investigated SNV G mutant allele is associated with worse responses to imatinib when compared to patients with the homozygous wild-type genotype [29, 57, 58, 29]. The same is true when analyzing the GG genotype of rs1695 in combination with the positive genotype of the *GSTM1* gene, showing that the variant is involved in imatinib response, and it is associated with variants in other genes [29].

Regarding, *SCL22A4*, like other genes in the SLC family, it is involved in the cellular elimination of various organic cations, such as drugs and various xenobiotics [59]. In relation to imatinib treatment, Angelini et al. [60] performed an analyses similar to ours, first analyzing several SNVs in candidate genes alone and obtained that individuals with the C allele of rs1050152 of *SLC22A4* respond better to treatment. Later, they made combinations between the *SLC22A4* gene variants and between other transporter genes (*SLC22A1* and *SLCO1A2*). Their result remained, stating the importance of this gene in imatinib. So far no other reports of interactions between *GSTP1* and *SLC22A4* genes have been found in the literature.

***BCL2L11, ABCB1* and *CYP2A6***

The rs724710 variant in the *BCL2L11* gene showed significant interaction with two different genes: *ABCB1* (rs1128503 and rs1045642) and *CYP2A6.*

The *BCL2L11* (Bcl-2-like 11) gene – also known as *BIM* – expresses a protein with pro-apoptotic action which modulates immune response [61, 62, 63]. Variants of the BCL2L11 gene have been linked with resistance to cancer treatments specifically targeted, as in lung cancer [64], Chronic Lymphocytic Leukemia and Acute Myeloid Leukemia [63].

Deletion of intron 2 in this gene is the target of studies associating it with CML treatment [65, 66, 67]. The polymorphism investigated in this work (rs724710 T>C) is an intronic SNV, and Augis and colleagues found that the T allele of this variant is more frequent in patients with no response to imatinib, and it is associated with a delay in the achievement of molecular response. In addition, the variant was also related to decreased basal mRNA expression in normal blood cells [68].

Otherwise, our results showed that the CC genotype is related to treatment resistance. The GTEx website (https://gtexportal.org/home/) points out that it is the homozygous mutant (CC) genotype is related to a decreased expression of the gene, which would lead to a less efficient protein and, consequently, to the permanence of the leukemic cells and treatment resistance. It is known that leukemic cells with decreased BIM expression are not good responders to TKIs [69, 70, 71].

Regarding the interaction between genes, Tsubaki et al. [72] reported that, in multiple myeloma cells, overexpression of MDR1 (expressed by *ABCB1*) and decreased expression of *BIM* (product of the *BCL2L11* gene) lead to resistance to multiple chemotherapeutic treatments. This information demonstrates that the downregulation of proteins in these genes and their interactions may affect the overall drug response.

About the *CYP2A6* gene, it is part of the cytochrome P450 superfamily of metabolizing enzymes [73, 74, 75, 76]. This SNV rs28399433 is an A>C exchange, located in the promoter region of the gene and disrupting the TATA box region, which results in decreased gene expression and affects the activity level of the expressed protein [77, 78]. This variant is also called CYP2A6*9.

This SNV has been related to the response of various treatments. Patients homozygous for the CYP2A6*9 variant are resistant to treatment for head and neck carcinoma [79] and have higher plasma efavirenz concentrations than individuals without this variant [78]. This information adds to our findings, as it reveals that the presence of mutant variant leads to impaired response to cancer treatments.

As for the interaction between *BCL2L11* and *CYP2A6*, this is the first study to report such information; however, considering the importance of both genes separately, the sum of their influence on the cell could result in significant impairment to treatment response.

***SLC29A1* and *ABCG2***

The *SLC29A1* gene (rs747199) has an interaction with the *ABCG2* gene (rs12505410 and rs2725252). There are no studies specifically demonstraing the interaction of these two genes, however, as they are both transporters, they may together influence these drug responses [80, 81].

The *ABCG2* gene – located in region 22 of the long arm of chromosome 4 – encodes the BCRP protein, which is responsible for transporting xenobiotics, including drugs, and has therefore been associated with resistance to treatments, especially chemotherapy. As with other

transporter genes, studies have shown that polymorphisms in this gene can alter drug delivery and treatment efficiency [31, 82, 83, 84].

Regarding the rs12505410 polymorphism and resistance to imatinib, Delord and colleagues (2013) [85] demonstrated the association between the rs12505410 and others variants with higher molecular response, thus patients with at least one copy of G-G genotype in the haplotype have better responses to treatment than patients with other genotypes. Thus, we highlight the potential of this finding to work as molecular marker while evaluating imatinib response.

The *SLC29A1* gene expresses a transmembrane glycoprotein that performs nucleoside transport [85]. Genetic alterations in this gene are related to treatment resistance in ALL [86], gastric cancer [87] and breast cancer [88].

Regarding CML, Leisewitz et al. [89] reported that mouse cells with the BCR-ABL mutation treated with imatinib showed reduced *SLC29A1* protein activity and expression of its messenger RNA. This finding was corroborated by Damaraju et al. [90], who observed that imatinib does decrease the gene expression. The SNV rs747199 decreases the gene's messenger RNA expression, which could imply loss of response over time [91].

**_ULK3_ and _SLCO1A2_**

The *SLCO1A2* gene is a member of the SLC family, which expresses passive, ion-dependent, exchanger transporter proteins [45]. Studies highlight that genetic variants in *SLCO1A2* may result in different protein activities and may modulate the uptake of xenobiotics [93, 94, 95, 96].

The SNVs rs4148977 and rs4148978 of the *SLCO1A2* gene were analyzed in this research and showed significance in interaction with the *ULK3* gene (rs2290573). They are located in the promoter region of the gene and in linkage disequilibrium with each other [97, 98].

These variants have been investigated in relation to imatinib concentration in *in vitro* [99] and *in vivo* studies. Yamakawa et al. [97] found that the SNVs rs3764043 (-361G>A) and rs4148977 (-1105G>A) / rs4148978 (-1032G>A) influence imatinib uptake in CML patients. Also, other markers in this gene have also been related to plasma concentration of imatinib [100] and to better response in CML treatment [96], thus revealing the influence of these markers on drug interaction and response.

We reinforce that this *ULK3* gene variant was significant both separately and in interactions, confirming its influence on secondary imatinib resistance in the treatment of CML.

## CONCLUSION

For the admixed population from Brazil northern region, the *ULK3* gene has an association with secondary resistance to this standard treatment for CML, and we also find that the combination of variants in several genes involved in the uptake, distribution, metabolism and excretion of imatinib has also been associated with this type of resistance.

Considering the plural cellular environment, genes and proteins do not act in isolation, there are extensive drug metabolization pathways, thus the interaction of polymorphisms is a feasible scenario. Therefore, the interaction between variants is pointed as an important factor in modulating the response to imatinib treatment in CML.

The use of SNVs as response predictors in the CML treatment may be an important tool to define the best therapy for patients with resistance not due to alterations in the BCR-ABL gene. Predictability can lead to the best therapeutic choice for oncology patients, resulting in individualized clinical practice, better quality of life for the patient, and reduced treatment costs.

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102.

## Supplementary material

Table 1. Selected variants genetics to analysis of association

| Gene | Variant | Alleles | Function | Amino acid change | Association (*pharmGKB*) |
|---|---|---|---|---|---|
| *ABCB1* | rs1045642 | A > T/ A > G | Synonymous | Ile1145Ile | Efficacy |
| *ABCB1* | rs1128503 | A > G | Synonymous | Gly412Gly | Efficacy |
| *ABCC2* | rs717620 | C > T | 5 UTR | *** | Toxicity |
| *ABCC4* | rs9561765 | G > A | Intronic | *** | Efficacy |
| *ABCC4* | rs9524885 | T>C | Intronic | *** | Efficacy |
| *ABCG2* | rs2231142 | G > T | Missense | Gln141Lys | Metabolism |
| *ABCG2* | rs2725252 | C > A | Intronic | *** | Efficacy |

| *ABCG2* | rs12505410 | T > G | Intronic | *** | Efficacy |
|---|---|---|---|---|---|
| *ABCG2* | rs13120400 | T > C | Intronic | *** | Efficacy |
| *ABCG2* | rs1061018 | A > G | Missense | Phe208Ser | Efficacy |
| *ABCG2* | rs41282401 | C > G | Missense | Asp296His | Efficacy |
| *ABCG2* | rs45605536 | C > T | Missense | Ala528Thr | Efficacy |
| *ABCG2* | rs2231137 | C > T | Missense | Val12Met | Efficacy |
| *BCL2L11* | rs724710 | T > C | 3' UTR | Ile95Ile | Efficacy |
| *CYP2A6* | rs28399433 | A > C | 5 Flanking | *** | Metabolism |
| *CYP2A6* | rs8192726 | C > A | Intronic | *** | Metabolism |
| *CYP3A4* | rs2740574 | C > T | 5' Flanking | *** | Toxicity |
| *CYP3A5* | rs776746 | T > C | Intronic | *** | Efficacy |
| *GSTP1* | rs1695 | A > G | Missense | Ile105Val | Efficacy |
| *SLC22A1* | rs683369 | G > C | Missense | Leu160Phe | Efficacy/ Metabolism |
| *SLC22A1* | rs2282143 | C > T | Missense | Pro341Leu | Efficacy |
| *SLC22A4* | rs1050152 | C > T | Intronic | Leu503Phe | Efficacy |
| *SLC22A5* | rs2631372 | G > C | Intronic | *** | Efficacy |
| *SLC22A5* | rs2631367 | C > G | Intronic | *** | Efficacy |
| *SLC22A7* | rs4149178 | A>G | Intronic | *** | Efficacy |
| *SLC29A1* | rs747199 | G > C | Intronic | *** | Efficacy |
| *SLCO1A2* | rs3764043 | C > T | 5' Flanking | *** | Metabolism |
| *SLCO1A2* | rs4148978 | C > T | 5' Flanking | *** | Metabolism |
| *SLCO1A2* | rs4148977 | C > T | 5' Flanking | *** | Metabolism |
| *ULK3* | rs2290573 | G > A | Intronic | *** | Efficacy |

Table 2. Characterization of *ABL1* gene variants selected as a control method

| **Gene** | **Variant** | **Alleles** | **Function** | **Amino acid change** | **Association (*pharmGKB*)** |
|---|---|---|---|---|---|
| *ABL1* | rs1057519771 | G>C | Missense | Val299Leu | Imatinib resistance |
| *ABL1* | rs121913452 | T>A / T>C / T>G | Missense | Phe359Ile/ Leu/ Val | Imatinib resistance |
| *ABL1* | rs1057519772 | A>G | Missense | Thr315Ala | Imatinib resistance |
| *ABL1* | rs121913454 | A>G | Missense | His396Arg | Imatinib resistance |
| *ABL1* | rs121913461 | T>C | Missense | Tyr253His/ Tyr272His | Imatinib resistance |
| *ABL1* | rs121913457 | T>C | Missense | Met351Thr | Imatinib resistance |
| *ABL1* | rs121913448 | G>A | Missense | Glu255Lys | Imatinib resistance |
| *ABL1* | rs121913459 | C > T | Missense | Thr315Ile | Imatinib resistance |

Table 3. Genotype distribution and quality control of the polymorphisms.

| Gene/rs | Genotype | | | | | Hardy-Weinberg Equilibrium |
|---|---|---|---|---|---|---|
| | Most Frequent Alelle | Nucleotide Change | AA[1] | Aa[2] | Aa[3] | p-value |
| **ABCB1 rs1045642** | G | A>T / A>G | 40 | 66 | 23 | 0.721 |
| **ABCB1 rs1128503** | G | A>G | 52 | 61 | 16 | 0.849 |
| **ABCC2 rs717620** | C | C>T | 105 | 22 | 2 | 0.614 |
| **ABBC4 rs9524885** | C | T>C | 66 | 56 | 7 | 0.372 |
| **ABCC4 rs9561765** | G | G>A | 120 | 9 | - | 1.000 |
| **ABCG2 rs2231142** | G | G>T | 100 | 27 | 2 | 1.000 |
| **ABCG2 rs2725252** | C | C>A | 40 | 65 | 24 | 0.859 |
| **ABCG2 rs12505410** | T | T>G | 60 | 58 | 11 | 0.681 |
| **ABCG2 rs13120400** | T | T>C | 94 | 33 | 2 | 1.000 |
| **ABCG2 rs1061018** | A | A>G | 129 | - | - | NA |
| **ABCG2 rs41282401** | C | C>G | 129 | - | - | NA |
| **ABCG2 rs45605536** | C | C>T | 128 | 1 | - | 1.000 |
| **ABCG2 rs2231137** | C | C>T | 88 | 40 | 1 | 0.193 |
| **BCL2L11 rs724710** | T | T>C | 34 | 64 | 31 | 1.000 |
| **CYP2A6 rs28399433** | A | A>C | 103 | 26 | - | 0.361 |
| **CYP2A6 rs8192726** | C | C>A | 117 | 12 | - | 1.000 |
| **CYP3A4 rs2740574** | T | C>T | 82 | 43 | 4 | 0.781 |
| **CYP3A5 rs776746** | C | T>C | 60 | 61 | 8 | 0.205 |
| **GSTP1 rs1695** | A | A>G | 43 | 59 | 27 | 0.474 |
| **SLC22A1 rs683369** | C | G>C | 109 | 17 | 3 | 0.059 |
| **SLC22A1 rs2282143** | C | C>T | 119 | 10 | - | 1.000 |
| **SLC22A4 rs1050152** | C | C>T | 67 | 53 | 9 | 0.827 |
| **SLC22A5 rs2631372** | G | G>C | 62 | 57 | 10 | 0.674 |
| **SLC22A5 rs2631367** | G | C>G | 51 | 63 | 15 | 0.570 |
| **SLC22A7 rs4149178** | A | A>G | 74 | 52 | 3 | 0.125 |
| **SLC29A1 rs747199** | G | G>C | 98 | 26 | 5 | 0.071 |
| **SLCO1A2 rs3764043** | C | C>T | 112 | 17 | - | 1.000 |

| SLCO1A2 rs4148978 | C | C>T | 73 | 49 | 7 | 1.000 |
|---|---|---|---|---|---|---|
| **SLCO1A2 rs4148977** | C | C>T | 73 | 48 | 7 | 1.000 |
| **ULK3 rs2290573** | G | G>A | 38 | 62 | 29 | 0.724 |
| **ABL1 rs1057519771** | G | G>C | 129 | - | - | NA |
| **ABL1 rs121913452** | T | T>A / T>C / T>G | 128 | - | - | NA |
| **ABL1 rs1057519772** | A | A>G | 129 | - | - | NA |
| **ABL1 rs121913454** | A | A>G | 129 | - | - | NA |
| **ABL1 rs121913461** | T | T>C | 129 | - | - | NA |
| **ABL1 rs121913457** | T | T>C | 128 | 1 | - | 1.000 |
| **ABL1 rs121913448** | G | G>A | 258 | - | - | NA |
| **ABL1 rs121913459** | C | C>T | 128 | 1 | - | 1.000 |

[1]AA: Most frequent homozygote; [2]Aa: Heterozygote; [3]aa: Less frequent homozygote

Table 4. Odds ratio and genotype distributions of the polymorphisms not statistically significant between the patients with response and the patients with secondary resistance.

| **Genotype** | **Responders (%)** | **Secondary resistance (%)** | **OR (95% CI)** | **Lower** | **Upper** | **p-value** |
|---|---|---|---|---|---|---|
| *ABCB1* rs1045642[1] | | | | | | |
| GG + AG | 63 (81.8) | 26 (92.9) | 1.00 | | | 0.137 |
| AA | 14 (18.2) | 2 (7.1) | 0.35 | 0.07 | 1.63 | |
| *ABCB1* rs1128503[1] | | | | | | |
| GG + GA | 66 (85.7) | 26 (92.9) | 1.00 | | | 0.301 |
| AA | 11 (14.3) | 2 (7.1) | 0.46 | 0.10 | 2.23 | |
| *ABCC2* rs717620[1] | | | | | | |
| CC + TC | 76 (98.7) | 27 (96.4) | 1.00 | | | 0.478 |
| TT | 1 (1.3) | 1 (3.6) | 2.81 | 0.17 | 46.58 | |
| *ABCC4* rs9561765[2] | | | | | | |
| GG | 72 (93.5) | 26 (92.9) | 1.00 | | | 0.906 |
| GA | 5 (6.5) | 2 (7.1) | 1.11 | 0.2 | 6.06 | |
| *ABCC4* rs9524885[1] | | | | | | |
| CC + CT | 73 (94.8) | 26 (92.9) | 1.00 | | | 0.709 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CT | 4 (5.2) | 2 (7.1) | 1.40 | 0.24 | 8.12 | |
| *ABCG2* | | | | | | |
| rs2231142[1] | | | | | | |
| GG + GT | 76 (98.7) | 28 (100.0) | 1.00 | | | 1.000 |
| TT | 1 (1.3) | 0 (0.0) | 0.00 | 0.00 | 0.00 | |
| *ABCG2* | | | | | | |
| rs2725252[1] | | | | | | |
| CC + CA | 62 (80.5) | 23 (82.1) | 1.00 | | | 0.850 |
| AA | 15 (19.5) | 5 (17.9) | 0.90 | 0.29 | 2.75 | |
| *ABCG2* | | | | | | |
| rs12505410[1] | | | | | | |
| TT + GT | 71 (92.2) | 27 (96.4) | 1.00 | | | 0.415 |
| GG | 6 (7.8) | 1 (3.6) | 0.44 | 0.05 | 3.81 | |
| *ABCG2* | | | | | | |
| rs13120400[1] | | | | | | |
| TT + CT | 75 (97.4) | 28 (100.0) | 1.00 | | | 1.000 |
| CC | 2 (2.6) | 0 (0.0) | 0.00 | 0.00 | 0.00 | |
| *ABCG2* | | | | | | |
| rs45605536[2] | | | | | | |
| CC | 76 (98.7) | 28 (100.0) | 1.00 | | | 1.00 |
| CT | 1 (1.3) | 0 (0.0) | 0.00 | 0.00 | 0.00 | |
| *ABCG2* | | | | | | |
| rs2231137[1] | | | | | | |
| CC + TC | 76 (98.7) | 28 (100.0) | 1.00 | | | 1.000 |
| TT | 1 (1.3) | 0 (0.0) | 0.00 | 0.00 | 0.00 | |
| *BCL2L11* | | | | | | |
| rs724710[1] | | | | | | |
| TT + CT | 60 (77.9) | 21 (75.0) | 1.00 | | | 0.754 |
| CC | 17 (22.1) | 7 (25.0) | 1.18 | 0.43 | 3.23 | |
| *CYP2A6* | | | | | | |
| rs28399433[2] | | | | | | |
| AA | 60 (77.9) | 23 (82.1) | 1.00 | | | 0.634 |
| AC | 17 (22.1) | 5 (17.9) | 0.77 | 0.25 | 2.32 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *CYP2A6* | | | | | | |
| rs8192726[2] | | | | | | |
| CC | 69 (89.6) | 24 (85.7) | 1.00 | | | 0.586 |
| CA | 8 (10.4) | 4 (14.3) | 1.44 | 0.40 | 5.21 | |
| *CYP3A4* | | | | | | |
| rs2740574[1] | | | | | | |
| TT + CT | 74 (96.1) | 27 (96.4) | 1.00 | | | 0.938 |
| CC | 3 (3.9) | 1 (3.6) | 0.91 | 0.09 | 9.16 | |
| *CYP3A5* rs776746[1] | | | | | | |
| CC + CT | 73 (94.8) | 27 (96.4) | 1.00 | | | 0.722 |
| TT | 4 (5.2) | 1 (3.6) | 0.68 | 0.07 | 6.32 | |
| *GSTP1* rs1695[1] | | | | | | |
| AA + GG | 59 (76.6) | 23 (82.1) | 1.00 | | | 0.539 |
| AG | 18 (23.4) | 5 (17.9) | 0.71 | 0.24 | 2.14 | |
| *SLC22A1* | | | | | | |
| rs683369[1] | | | | | | |
| CC + GC | 75 (97.4) | 28 (100.0) | 1.00 | | | 1.000 |
| GG | 2 (2.6) | 0 (0.0) | 0.00 | 0.00 | 0.00 | |
| SLC22A1 | | | | | | |
| rs2282143[2] | | | | | | |
| CC | 70 (90.9) | 25 (89.3) | 1.00 | | | 0.804 |
| CT | 7 (9.1) | 3 (10.7) | 1.20 | 0.29 | 5.00 | |
| *SLC22A4* | | | | | | |
| rs1050152[1] | | | | | | |
| CC + CT | 71 (92.2) | 26 (92.9) | 1.00 | | | 0.911 |
| TT | 6 (7.8) | 2 (7.1) | 0.91 | 0.17 | 4.80 | |
| *SLC22A5* | | | | | | |
| rs2631372[1] | | | | | | |
| GG + CG | 72 (93.5) | 25 (89.3) | 1.00 | | | 0.485 |
| CC | 5 (6.5) | 3 (10.7) | 1.73 | 0.38 | 7.76 | |
| *SLC22A5* | | | | | | |
| rs2631367[1] | | | | | | |
| GG + GC | 67 (87.0) | 26 (92.9) | 1.00 | | | 0.384 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CC | 10 (13.0) | 2 (7.1) | 0.52 | 0.11 | 2.51 | |
| *SLC22A7* | | | | | | |
| rs4149178[1] | | | | | | |
| AA + AG | 75 (97.4) | 28 (100.0) | 1.00 | | | 1.000 |
| GG | 2 (2.6) | 0.0 (0.00) | 0.00 | 0.00 | 0.00 | |
| *SLC29A1* | | | | | | |
| rs747199[1] | | | | | | |
| GG + CG | 75 (97.4) | 26 (92.9) | 1.00 | | | 0.310 |
| CC | 2 (2.6) | 2 (7.1) | 2.88 | 0.39 | 21.53 | |
| *SLCO1A2* | | | | | | |
| rs3764043[2] | | | | | | |
| CC | 69 (89.6) | 23 (82.1) | 1.00 | | | 0.319 |
| CT | 8 (10.4) | 5 (17.9) | 1.87 | 0.56 | 6.31 | |
| *SLCO1A2* | | | | | | |
| rs4148977[1] | | | | | | |
| CC + CT | 71 (92.2) | 27 (96.4) | 1.00 | | | 0.415 |
| TT | 6 (7.8) | 1 (3.6) | 0.44 | 0.05 | 3.81 | |
| *SLCO1A2* | | | | | | |
| rs4148978[1] | | | | | | |
| CC + CT | 71 (92.2) | 27 (96.4) | 1.00 | | | 0.415 |
| TT | 6 (7.8) | 1 (3.6) | 0.44 | 0.05 | 3.81 | |

[1]Recessive model; [2]Codominant model; *The SNVs rs1061018 (*ABCG2*) and rs41282401 (*ABCG2*) are monomorphics because of that they are not in the table

Table 5. Odds ratio and genotype distributions of the polymorphisms of *ABL1* gene between the patients with response and the patients with secondary resistance.

| **Genotype[1]** | **Responders (%)** | **No responders (%)** | **OR (95% CI)** | **Lower** | **Upper** | **p-value** |
|---|---|---|---|---|---|---|
| *ABL1* rs121913457 | | | | | | |
| TT | 76 (98.7%) | 28 (100.0%) | 1.00 | 0 | NA | 1.00 |
| CT | 1 (1.3%) | 0 (0.0%) | 0.00 | 0 | NA | |

[1]Codominant model; *The SNVs rs1057519771, rs1057519772, rs121913448, rs121913452, rs121913454, rs121913459 and rs121913461 are monomorphics because of that they are not in the table

# 5. DISCUSSÃO

Os artigos produzidos por este trabalho nos permitem observar que alterações genéticas do tipo SNV podem influenciar diversos aspectos de uma doença, desde a susceptibilidade até a resposta do indivíduo ao tratamento (GUO et al., 2019; FRIKHA et al., 2020). Desta maneira, investigar biomarcadores em genes de interesse e esclarecer seu papel no contexto geral do organismo é extremamente relevante e um campo de pesquisa que está em pleno desenvolvimento.

Ao reunirmos os achados nos artigos que compõem essa pesquisa, podemos notar como o conjunto de variantes genéticas e suas interações entre si podem influenciar fortemente a saúde global do indivíduo, se assemelhando com o que ocorre *in vivo* no ambiente celular onde alterações e interações ocorrem de forma somativa e simultaneamente (AU et al., 2014; ANKATHIL et al., 2018; DE SANTIS et al., 2022). O resumo dos resultados obtidos pode ser observado no quadro 4.

**Quadro 4** - Resumo dos resultados encontrados nos artigos

| Genes | Resultados encontrados |
|---|---|
| *CYP2A6 e ABCC4* | Efeito protetor para susceptibilidade |
| *ATIC e ARID5B* | Falha terapêutica ao decorrer do tratamento |
| *ULK3* | Resistência secundária |
| Interações de SNVs em genes de transporte e metabolismo do imatinibe | Resistência secundária |

**Fonte:** De autoria própria.

No capítulo I, obtivemos como resultado que as variantes rs2372536 do gene *ATIC* e rs10821936 do gene *ARID5B* relacionados com a falha terapêutica do imatinibe. E embora não sejam genes que interagem diretamente com o medicamento, notou-se que podem modular a resposta ao tratamento, influenciando na resistência à terapia da LMC. Destacamos que essas variantes nunca foram rastreadas para esta condição antes.

Já no capítulo II foi evidenciado que o SNV rs2290573 do gene *ULK3* envolvido na resistência secundária ao tratamento da LMC. Este gene já havia sido relacionado à resposta ao tratamento com imatinibe, assim essa pesquisa reforçou o que já vem sendo discutido na literatura e acrescentou uma nova perspectiva com a análise de interação que será abordada abaixo (DRESSMAN et al., 2004; DELORD et al., 2013).

As diversas interações entre as variantes investigadas dos genes de transporte e metabolismo do imatinibe se apresentaram como relevante para a resistência secundária. E aqui ressaltamos a importância da interação das alterações genéticas dentro do ambiente celular, pois separadamente a maior parte destas variantes não apresentaram significância mas ao reunirmos elas para análise podemos observar a influência que exercem sob a resposta ao tratamento. Assim, observar os efeitos somatórios das alterações genéticas e realizar a análise de diversos marcadores em conjunto é relevante para pesquisas que relacionam esses tipos de variações com características clínicas (ANGELINI et al., 2013; WANG et al., 2018; DELMOND et al., 2021).

Em resumo, com base nas informações apresentadas nesta tese temos a visão de que cada artigo agrega conhecimento de maneira única para que possamos tentar elucidar a resistência à terapia ao tratamento com imatinibe na LMC e assim buscar maneiras de intervir de maneira precoce e auxiliar a terapia para que mais pacientes possam ser beneficiados.

# 6. CONCLUSÃO

Diante disso, concluímos que a avaliação de variantes genéticas é relevante para a compreensão e predição da falha terapêutica ao tratamento da LMC com o imatinibe. Destacando que a investigação de marcadores genéticos ligados à via não dependente de *BCR-ABL* é importante para entender o processo de resistência de maneira mais ampla, o mesmo acontece com as interações que auxiliam a compreensão de uma visão mais abrangente acerca desse processo já que podem influenciar fortemente a resistência à terapia.

A utilização de biomarcadores genéticos preditivos antes do início do tratamento com imatinibe para pacientes com Leucemia mieloide crônica é importante e significativo para o estabelecimento de uma conduta terapêutica mais adequada pois beneficia a qualidade de vida do paciente possibilitando uma resposta mais eficaz e mais rápida pois a escolha do medicamento será assertiva.

Por fim, destacamos que este trabalho pode ser útil para o estabelecimento de futuras políticas de saúde públicas que visem melhorar o atendimento de paciente com LMC e tratados com imatinibe.

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**ANEXO I: Polymorphisms in the *CYP2A6* and *ABCC4* genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population**

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ORIGINAL ARTICLE

# Polymorphisms in the CYP2A6 and ABCC4 genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population

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**Abstract**

**Background:** Susceptibility to Chronic Myeloid Leukemia (CML) may be modulated by genetic variables. However, the majority of previous investigations have focused on genetically homogeneous populations, resulting in a lack of evidence on how genetic factors may influence the development of CML in miscegenated populations. We analyzed 30 polymorphisms in genes related to DNA repair, folate metabolism, transmembrane transport, xenobiotic metabolism, and pyrimidine synthesis in relation to their potential role in the susceptibility of the individual to CML.

**Methods:** This case-control study included 126 healthy individuals and 143 patients diagnosed with CML from the admixed population of the Brazilian Amazon. The samples were genotyped by real-time PCR and the genetic ancestry analysis was based on a panel of 61 ancestry informative markers.

**Results:** The results indicated a protective effect against the development of CML in carriers of the C allele of the rs28399433 (*CYP2A6*) gene and the CC genotype of the rs3742106 (*ABCC4*) gene.

**Conclusion:** Our findings suggest that the rs3742106 (*ABCC4*) and rs28399433 (*CYP2A6*) polymorphisms may modulate susceptibility to CML in a population of the Brazilian Amazon region.

KEYWORDS

*ABCC4*, ancestry, chronic myeloid leukemia, *CYP2A6*, genetic susceptibility

## 1 | INTRODUCTION

The carcinogenesis of Chronic Myeloid Leukemia (CML) is complex and multifactorial (Li et al., 2014). The principal genetic hallmark of CML is the *BCR-ABL1* oncogene, originated by the t(9;22) translocation, which fuses *ABL1* on chromosome 9q34 to *BCR* on chromosome 22q11 (Langabeer, 2013). This encodes the chimeric protein BCR-ABL, a constitutively active tyrosine kinase that drives the pathogenesis of CML (Egan & Radich, 2016).

The etiology of CML is still not completely elucidated. Some studies have reported that environmental factors may be involved

The authors Natasha Monte and Karla B C C Pantoja share the first authorship.
[Correction added on July 2, 2021, after first Online publication: The first authorship footnote has been included.]

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in the development of the disease, but this is not conclusive (Bispo et al., 2020; Musselman et al., 2013). The importance of the expression of *BCR-ABL* in the onset and progression of CML is well established, however, although other genes may also be involved in its development. For example, Ferri et al., (2019) found an association with CML risk in the case of the A allele of the rs12573787 polymorphism of the phosphatase and tensin homolog gene (*PTEN*), a tumor suppressor gene involved in the modulation of cell proliferation and apoptosis.

To better understand the genetic factors involved in the etiology of CML, we investigated 30 polymorphisms in genes of the carcinogenic metabolism involved in susceptibility to different types of cancer, including CML (Campa et al., 2012; Özten et al., 2012; Weich et al., 2016). These genes encode DNA repair proteins (Coskunpinar et al., 2015; Damineni et al., 2014), transmembrane carriers (Campa et al., 2012; Pereira et al., 2016), and endogenous and xenobiotic-metabolizing enzymes (He et al., 2014; Islam et al., 2013; Zhao et al., 2016).

The genetic variability of the target population is an important consideration for susceptibility studies, given that groups with distinct ancestry may present substantial differences in their allelic frequencies. Given this, the findings on a specific population may not be applicable to other groups that have a distinct genetic composition (Carvalho et al., 2015; Yu & Chen, 2012;). One clear example here is the Brazilian population, which is one of the most genetically heterogeneous groups found anywhere in the world, with principal contributions from three parental groups: Europeans, Amerindians, and Africans (Carvalho et al., 2015). The study of highly admixed populations is paramount to the comprehension of the influence of genetic polymorphisms on the predisposition of the individual to develop complex diseases, such as CML.

Given these considerations, the present study evaluated the association between the polymorphisms of key genes (in the transport and metabolism pathways) and susceptibility to CML in a population with a high degree of genetic admixture from the Brazilian Amazon. The key genes evaluated in this study and their OMIM access numbers are: FPGS (136510), ABCC2 (601107), ABCC4 (605250), ABCB1 (171050), ABCG2 (603756), SLC29A1 (602193), SLC22A7 (604995), DPYD (612779), CYP2A6 (122720), UMPS (613891), MTHFR (607093), GGH (601509), RRM1 (180410), and TP53 (191170).

## 2 | MATERIAL AND METHODS

### 2.1 | Ethical compliance

The present study was approved by the Research Ethics Committees of the Oncology Research Nucleus, under protocol number 3.354.571/2019, and the Ophir Loyola Hospital, under protocol number 1.575.920/2016. All the participants signed a statement of informed consent.

### 2.2 | Case and controls

The participants in the present study were selected based on a retrospective case-control study design. Data and samples were collected from 269 individuals, of which, 143 were patients diagnosed with CML (case group) and 126 were cancer-free individuals (control group), both from the city of Belém, located in the Amazon region of Brazil.

The CML patients had been treated for a minimum of 5 years and a maximum of 15 years, and they all had well-documented laboratory and clinical data. These patients were being treated in the onco-hematology sector of the Ophir Loyola Hospital in Belém (Pará, Brazil). The control group consisted of elderly individuals (ages of between 60 and 75 years) with no clinical reports of any type of cancer. Some of these individuals had been diagnosed with chronic conditions, such as hypertension or diabetes.

### 2.3 | Selection of markers

Thirty Single Nucleotide Polymorphisms (SNPs) of 14 genes were chosen through a search of two databases, *The Human Gene Mutation Database* (www.hgmd.cf.ac.uk/) and *PharmGKB* (www.pharmgkb.org/). The markers were selected based on two principal criteria: (1) polymorphisms involved in pivotal intracellular metabolic activities, and (2) previous reports of the marker in associative studies found in the *Pubmed* (www.ncbi.nlm.nih.gov/) database, which identify the marker as a potential predictor of susceptibility to different types of cancer. The polymorphisms presented in Table 1 met these prerequisites, and were thus selected for the analyses presented here.

### 2.4 | Extraction and quantification of the DNA

The DNA was extracted using the commercial Axy Prep™ Blood Genomic DNA Miniprep kit (Axygen Biotechnology), according to the manufacture's recommendations. The concentration and purity of the DNA were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000; NanoDrop Technologies).

### 2.5 | Genotyping

The polymorphisms were genotyped by allelic discrimination using the TaqMan OpenArray Genotyping technology, which was run in a QuantStudio™ 12K Flex Real-Time PCR system (Applied Biosystems, Life Technologies), according to the manufacture's protocol.

**TABLE 1** Polymorphisms chosen after applying the criteria of selection of markers

| Gene | Pathway | Reference sequence (RefSeq) | SNP |
|---|---|---|---|
| *TP53* | DNA repair | NG_017013.2 | rs1042522 |
| *RRM1* | DNA repair | NG_027992.2 | rs1042927 |
| *RRM1* | | | rs12806698 |
| *MTHFR* | Folate metabolism | NG_013351.1 | rs1801131 |
| *MTHFR* | | | rs1801133 |
| *GGH* | Folate metabolism | NG_028126.1 | rs3758149 |
| *FPGS* | Folate metabolism | NG_023245.1 | rs4451422 |
| *ABCG2* | Transmembrane transporter | NG_032067.2 | rs2231142 |
| *ABCB1* | Transmembrane transporter | NG_011513.1 | rs1045642 |
| *ABCB1* | | | rs1128503 |
| *ABCC2* | Transmembrane transporter | NG_011798.2 | rs717620 |
| *ABCC4* | Transmembrane transporter | NG_050651.2 | rs4148551 |
| *ABCC4* | | | rs3741206 |
| *ABCC4* | | | rs9524885 |
| *SLC29A1* | Transmembrane transporter | NG_042893.1 | rs747199 |
| *SLC29A1* | | | rs760370 |
| *SLC22A7* | Transmembrane transporter | NC_000006.12 | rs2270860 |
| *SLC22A7* | | | rs4149178 |
| *DPYD* | Xenobiotic-metabolizing | NG_008807.2 | rs17116806 |
| *DPYD* | | | rs1801159 |
| *DPYD* | | | rs1801265 |
| *DPYD* | | | rs3918290 |
| *DPYD* | | | rs4970722 |
| *DPYD* | | | rs55886062 |
| *DPYD* | | | rs67376798 |
| *DPYD* | | | rs17376848 |
| *DPYD-AS1* | Xenobiotic-metabolizing | NC_000001.11 | rs1760217 |
| *CYP2A6* | Xenobiotic-metabolizing | NG_008377.1 | rs28399433 |
| *CYP2A6* | | | rs8192726 |
| *UMPS* | Pyrimidine synthesis | NG_017037.1 | rs1801019 |

## 2.6 | Quality control

The polymorphisms that were not in Hardy-Weinberg equilibrium or had at least 15% of missing genotypes were excluded from subsequent statistical analyses. Of the 30 markers selected initially, then only 13 polymorphisms met all the criteria for analysis (see Table S1 in the Appendix).

## 2.7 | Analysis of genetic ancestry

Genetic ancestry was analyzed according to Ramos et al., (2016), using 61 autosomal ancestry informative markers in three multiplex PCR reactions. The amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and the Gene Mapper ID v.3.2 software. The individual proportions of European, African, and Amerindian genetic ancestry were estimated using STRUCTURE v.2.3.3, assuming the contribution of three parental populations.

## 2.8 | Statistical analysis

The statistical analyses were run in RStudio v.3.6.1 (SNPassoc library). Differences in the categorical variable (sex) were tested using Pearson's Chi-square, while the quantitative variable (mean age) was evaluated using Student's *t*. The ancestry indices were compared between case and control groups using the Mann-Whitney test. Multiple logistic regressions were used to assess possible associations between the polymorphisms and susceptibility to CML, by estimating the odds ratios (ORs) and their 95% confidence intervals (CIs).

Sex and age were controlled for in this multivariate analysis, to avoid confounding associations. A significance level of $p < 0.05$ was considered for all the statistical analyses.

## 3 | RESULTS

### 3.1 | Description of the study population

The results of the epidemiological analyses are presented in Table 2. Women predominated in the control group, accounting for 66.7% of all individuals, whereas men dominated the CML patients, with 63.7% of the total. There was a significant difference ($p < 0.001$) between the case and control groups in the distribution of the sexes. The mean age of the case group (47.5 years) was also significantly lower ($p < 0.001$) than that of the control group (66.0 years). Given these differences between the two groups, both age and sex were controlled for in the subsequent analyses, in order to minimize their interference in the assessment of the genetic data.

The analysis of the genetic ancestry of the participants revealed a mostly European ancestry in both groups, being 46.3% in the case group and 44.2% in the control group, followed by Amerindian (31.0% in patients and 30.5% in the control individuals), and African ancestry (22.6% in the case group and 25.4% in the control). There was no significant variation ($p > 0.05$) in the ancestral makeup of the two groups.

### 3.2 | Genotype distribution and risk estimates

Table 3 shows the distribution of the genotypes that varied significantly between the case and control groups, in in addition to their ORs, with regard to the potential susceptibility to CML. Significant differences were found in the cases of the rs28399433 polymorphism of the *CYP2A6* gene and the rs3742106 polymorphism of the *ABCC4* gene.

Individuals with the C allele of the rs28399433 polymorphism of the *CYP2A6* gene, either homozygously or heterozygously, presented a significant protective effect against the development of CML ($p = 0.008$; OR = 0.33; 95% CI = 0.14–0.78). Similarly, carriers of the CC genotype of the rs3742106 variant of the *ABCC4* gene also presented a significantly reduced susceptibility to CML ($p = 0.020$; OR = 0.30; 95% CI = 0.10–0.88), in comparison with the other genotypes.

None of the other polymorphisms presented any significant variation between case and control groups. The distribution of the genotypes in the case and control groups is shown in the Appendix (Table S2).

**TABLE 2** Epidemiological data of the CML patients and the control group

| Variable | CML (%) | Control (%) | *p*-value |
|---|---|---|---|
| Total | 143 | 126 | |
| Age (years) | 47.41 ± 14.93 | 66.02 ± 4.45 | <0.001 |
| Sex (%) | | | |
| Men | 79 (63.7) | 42 (33.3) | <0.001 |
| Women | 45 (36.3) | 84 (66.7) | |
| Ancestry mean | | | |
| European | 0.463 ± 0.149 | 0.442 ± 0.161 | 0.268 |
| Amerindian | 0.310 ± 0.138 | 0.305 ± 0.147 | 0.572 |
| African | 0.226 ± 0.097 | 0.254 ± 0.147 | 0.517 |

## 4 | DISCUSSION

Carcinogenesis is a multistep process that involves both genetic and environmental risk factors. The presence of genetic polymorphisms in pivotal genes may affect the structure, function, stability or folding of the encoded proteins, which may initiate a carcinogenic event (Frikha et al., 2020; Zhang & Zhu, 2020). The present study evaluated the possible association of SNPs in genes of key cellular pathways, such as the transport of substances across cell membranes, DNA repair, the folate and xenobiotic metabolisms, and pyrimidine synthesis, with a predisposition to the development of CML.

Over the years, these polymorphisms have been associated with different cancers and other complex diseases. The *RRM1* gene, for example, has been related to a high degree of sensitivity to oncological chemotherapy (Yang et al., 2019; Zhao et al., 2012) and has been identified as a predictive factor in gemcitabine therapy in oncology patients (Jordheim et al., 2011). The *ABCG2* gene has also been associated with resistance to chemotherapy in the treatment of many types of cancer (Amawi et al., 2019; Robey et al., 2018). Polymorphisms of the *CYP* gene family are also known to modulate susceptibility to a number of different complex diseases (Daly, 2015). Up to now, however, there has been little research into possible associations between these variants and susceptibility to CML.

The results of the present study indicated that carriers of the C allele (whether homozygous or heterozygous) of the rs28399433 polymorphism of the *CYP2A6* gene are significantly less susceptible to the development of CML in comparison with the AA genotype. Previous studies have shown that the rs28399433 polymorphism, which is common in Asian populations, is located in the TATA box region, and is responsible for reducing gene transcription (Rodriguez-Antona et al., 2010).

**TABLE 3** Odds Ratios and genotype distribution of the statistically significant polymorphisms in the CML patients and the control group

| Genotype | Control (%) | CML (%) | *p*-value | OR (95% CI)[a] |
|---|---|---|---|---|
| *CYP2A6*[b] rs28399433 | | | | |
| AA | 107 (66.5) | 95 (83.3) | 0.008 | AC + CC vs. AA[c]: 0.33 (0.14–0.78) |
| AC | 45 (28.0) | 17 (14.9) | | |
| CC | 9 (5.6) | 2 (1.8) | | |
| *Allele* A | 107 (66.5) | 95 (83.3) | | |
| *Allele* C | 54 (33.5) | 19 (16.7) | | |
| *ABCC4*[e] rs3742106 | | | | |
| AA | 43 (29.7) | 43 (37.1) | 0.020 | CC vs. AA + AC[d]: 0.30 (0.10–0.88) |
| AC | 71 (49.0) | 55 (47.4) | | |
| CC | 31 (21.4) | 18 (15.5) | | |
| *Allele* A | 114 (78.6) | 98 (84.5) | | |
| *Allele* C | 31 (21.4) | 18 (15.5) | | |

[a]Multiple logistic regression adjusted by age and gender.

[b]*CYP2A6* RefSeq: NG_008377.1.

[c]Heterozygous genotype + mutant homozygous genotype *vs*. Wild homozygous genotype.

[d]Mutant homozygous genotype *vs*. Wild homozygous genotype + heterozygous genotype.

[e]*ABCC4* RefSeq: NG_050651.2.

The only previous study that has linked this variant to predisposition to cancer was that of Ezzeldin et al., (2018), who attempted to establish a relationship between this polymorphism and the risk of lung cancer in the population of Egypt. However, this variant occurred at a prohibitively low frequency in the study group (3.7%) to support the statistical analyses.

The association between other polymorphisms of the *CYP2A6* gene and cancer has also been evaluated in a number of other studies. For example, Song et al., (2009) found that the deletion of the entire *CYP2A6* gene (CYP2A6*4) resulted in a reduced risk of bladder cancer in Chinese smokers, while Coskunpinar et al., (2015) found that this same variant was linked to a decreased risk of lung cancer in a population from Bangladesh.

The present study also showed that the homozygous CC genotype of the rs3742106 polymorphism of the *ABCC4* gene confers a decreased susceptibility to the development of CML. It is important to note here that *ABCC4*, also known as the multi-drug resistance-associated protein 4, is an important member of the ATP-binding cassette transporter family, and is responsible for transporting a variety of endogenous and exogenous organic anions, of varying composition, out of the cell (Wen et al., 2015). Given its chemotherapeutic drug efflux capacity, *ABCC4* has been studied extensively in relation to drug resistance, in various types of cancer cells. Previous research has also shown that this gene influences the biology of the cancer cell.

For example, Zhao et al., (2014) found a high level of expression of ABCC4 in different lung cancer cell lines. In this study, the authors reported that the suppression of the expression of the ABCC4 messenger RNA (mRNA) resulted in a decrease in cell proliferation, probably due to the ability of ABCC4 to transport the molecules involved in cell signaling. Corroborating these findings, Chen et al., (2017) demonstrated in cell culture that the presence of the T allele of the rs3742106 polymorphism affects the regulatory role of the mRNA and thus decreases the expression of the ABCC4 protein.

A high level of ABCC4 expression has also been observed in aggressive primary neuroblastoma (Murray et al., 2017), the blast cells of adult patients with acute myeloid leukemia, and in acute childhood lymphoblastic leukemia (Copsel et al., 2011; Mesrian Tanha et al., 2017). Pereira et al., (2014) also found an association between other variants of the *ABCC4* gene, which were not evaluated in the present study, and colorectal carcinogenesis. Despite the existence of previous studies linking polymorphisms of the *ABCC4* gene with certain types of cancer, there has been no research on the relationship between the rs3742106 variant and a predisposition to neoplasms or other diseases.

As shown in the present study, the proteins expressed by these genes can be modulated by SNPs, which modifies their function. In the case of the *ABBC4* gene, this would affect the cell's efflux, while in the *CYP2A6* gene, the effect would be on the metabolizing activity of potential carcinogens (Ezzeldin et al., 2018; Rodriguez-Antona et al., 2010), which would contribute to the carcinogenic process.

In addition to the variation in the genotype frequencies of the study polymorphisms and their association with the risk

of CML, significant differences were also found in the mean age and the sexes of the case and control groups, but not in their ancestral makeup.

These findings indicate that CML occurs more frequently in men than in women, which is consistent with the findings of two studies in Bangladesh and Pakistan (Bhatti et al., 2012; Mottalib et al., 2014). It remains unclear, however, while there is a higher frequency of CML in men, with different studies suggesting social, behavioral, or even biological factors (Bortolheiro & Chiattone, 2008). Radivoyevitch et al., (2014) raised the hypothesis that men have more target cells at risk of developing CML than women.

The mean ages of the two groups were significantly different ($p < 0.001$; case = 47.41 ± 14.93 years; control = 66.02 ± 4.45 years). As the incidence of CML is known to increase with age, peaking between 55 and 60 years of age, the control group was selected specifically to include individuals of an older age.

## 5 | CONCLUSIONS

The results of the present study demonstrated that the rs28399433 (CYP2A6) and the rs3742106 (ABCC4) polymorphisms are associated with a protective effect against the development of CML in a highly miscegenated population from the Brazilian Amazon. This is the first study to associate genetic polymorphisms with a susceptibility to CML in an admixed population from the Brazilian Amazon region, which has a unique genetic background and may thus deviate from the patterns found in more genetically homogeneous populations.

The present study is the first to show an association between polymorphisms of the ABCC4 and CYP2A6 genes with a decreased predisposition for the development of CML. The findings of this study may provide important insights into the genetic predisposition of individuals to develop CML, although further research will be required to provide a more conclusive interpretation of the observed patterns.

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### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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# ANEXO II - Aprovação do comitê de ética em pesquisa

UFPA - NÚCLEO DE PESQUISA EM ONCOLOGIA DA UNIVERSIDADE FEDERAL DO PARÁ

Plataforma Brasil

## PARECER CONSUBSTANCIADO DO CEP

**DADOS DA EMENDA**

**Título da Pesquisa:** Desenvolvimento de um painel molecular de medicina de precisão envolvido na resposta a terapia com uso de Imatinibe em pacientes com Leucemia Mielóide Crônica

**Pesquisador:** Tereza Cristina de Brito Azevedo

**Área Temática:** Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

**Versão:** 4

**CAAE:** 51100515.4.0000.5634

**Instituição Proponente:** Núcleo de Pesquisa em Oncologia

**Patrocinador Principal:** Universidade Federal do Pará

**DADOS DO PARECER**

**Número do Parecer:** 4.062.662

| Tipo Documento | Arquivo | Postagem | Autor | Situação |
|---|---|---|---|---|
| Informações Básicas do Projeto | PB_INFORMAÇÕES_BÁSICAS_1143789_E1.pdf | 14/02/2020 12:14:32 | | Aceito |
| Outros | CARTA_RESPOSTA_AO_PARECER_EMENDA_2.pdf | 14/02/2020 12:03:34 | KARLA BEATRIZ CARDIAS CEREJA PANTOJA | Aceito |
| Projeto Detalhado / Brochura Investigador | Projeto_Completo_emenda_2.pdf | 14/02/2020 12:00:37 | KARLA BEATRIZ CARDIAS CEREJA PANTOJA | Aceito |
| TCLE / Termos de Assentimento / Justificativa de Ausência | TERMO_DE_CONSENTIMENTO_LMC_EMENDA_2.pdf | 14/02/2020 11:59:51 | KARLA BEATRIZ CARDIAS CEREJA PANTOJA | Aceito |
| Declaração de Pesquisadores | declaracao_de_vinculo_pesquisadora.pdf | 15/03/2016 17:32:41 | Tereza Cristina de Brito Azevedo | Aceito |
| Declaração de Instituição e Infraestrutura | carta_de_anuencia_hemopa.pdf | 15/03/2016 17:30:56 | Tereza Cristina de Brito Azevedo | Aceito |
| Declaração de Instituição e Infraestrutura | declaracao_de_instituicao.pdf | 10/11/2015 13:00:07 | Tereza Cristina de Brito Azevedo | Aceito |
| Folha de Rosto | Folha_de_rosto.pdf | 10/11/2015 12:58:30 | Tereza Cristina de Brito Azevedo | Aceito |

**Situação do Parecer:**
Aprovado

**Necessita Apreciação da CONEP:**
Não

BELEM, 01 de Junho de 2020

## ANEXO III - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

I - DADOS DE IDENTIFICAÇÃO DO PARTICIPANTE DA PESQUISA

1. NOME DO PARTICIPANTE: ______________________________________
DOCUMENTO DE IDENTIDADE Nº: ___________________ SEXO: M ( ) F ( )
DATA NASCIMENTO: _____/_____/_____

II - DADOS SOBRE A PESQUISA CIENTÍFICA:

1. TÍTULO DO PROTOCOLO DE PESQUISA: DESENVOLVIMENTO DE UM PAINEL MOLECULAR DE MEDICINA DE PRECISÃO ENVOLVIDO NA RESPOSTA A TERAPIA COM USO DE IMATINIBE EM PACIENTES COM LEUCEMIA MIELOIDE CRÔNICA

2. PESQUISADOR RESPONSÁVEL: TEREZA CRISTINA DE BRITO AZEVEDO

III - INFORMAÇÕES A (O) PARTICIPANTE
O (A) senhor (a) está sendo convidado (a) a participar do projeto de pesquisa: "Desenvolvimento de um painel molecular de medicina de precisão envolvido na resposta a terapia com uso de imatinibe em pacientes com Leucemia Mieloide Crônica", o qual será realizado no Hospital Ophir Loyola (HOL) e no Núcleo de Pesquisas em Oncologia (NPO) da Universidade Federal do Pará (UFPA).

IV – OBJETIVOS DA PESQUISA
A Leucemia Mielóide Crônica (LMC) é uma doença caracterizada pelo crescimento maligno das células que formam o sangue. Os pacientes com LMC são diagnosticados através de testes hematológicos nos quais realiza-se a pesquisa do cromossomo Philadelphia (alteração que indivíduos saudáveis não possuem) e que funciona como um indicador da doença. Estudos clínicos demostram que durante o tratamento, devido a variabilidade genética dos pacientes pode ocorrer uma variação na resposta e alguns pacientes podem apresentar resistência ao longo do tratamento. Desta forma busca-se analisar e identificar possíveis mutações e genes que estejam associados com a farmacogenética do imatinibe com o intuito de compreender melhor a LMC e o seu tratamento.

V – DESCRIÇÃO DOS PROCEDIMENTOS
A fim de alcançar o objetivo deste estudo será necessário a coleta de informações sobre seu estado clínico e resultados de exames de rotina solicitados pelo médico responsável, contidas no seu prontuário médico. Para as análises genéticas serão necessários 5 ml de sangue, em 1 tubo com EDTA, colhido com material estéril e descartável, por integrantes da equipe da pesquisa.

Convidamos V. Sa. a participar do estudo, lembrando que sua participação é voluntária (não havendo pagamento ou custo pela mesma). V. As. Pode solicitar a sua retirada da pesquisa a qualquer momento, e tal decisão não prejudicará a conduta clínica nem o seu atendimento e acompanhamento assistencial.

VI- DESCONFORTOS E RISCOS ESPERADOS DECORRENTES DO PROCEDIMENTO

Durante a coleta de sangue, pode haver uma leve dor e um pequeno hematoma pode aparecer, se isto ocorrer espera-se que desaparecerá após alguns dias da coleta. Para evitar qualquer

desconforto, a equipe de coleta foi treinada para realizar o procedimento e está comprometida em minimizá-los, assim como garantir as devidas medidas de segurança.

VII - BENEFÍCIOS QUE PODERÃO SER OBTIDOS

Os participantes da pesquisa não terão benefícios diretos mas a amostra de sangue cedida por eles contribuirá para o desenvolvimento desta pesquisa que busca otimizar o tratamento da LMC para que, depois dos resultados obtidos, outros pacientes futuramente possam usufruir de um tratamento ainda mais eficaz e contribua na construção de novos conhecimentos nas áreas de farmacogenética e da Leucemia Mielóide Crônica, trazendo benefícios para a comunidade científica.

VIII – CONFIDENCIALIDADE, INFORMAÇÕES E SIGILO

A confidencialidade e sigilo de suas informações será mantida e sua identidade será preservada durante toda a pesquisa, sendo que somente os membros da pesquisa terão acesso aos registros. Os dados serão armazenados por codificações, e o acesso ao banco de dados será mediante senha, liberada somente para os pesquisadores.

A sua participação neste estudo é voluntária, tendo o direito de retirar-se a qualquer momento. A recusa ou desistência da participação nesse estudo não irá prejudicar seu acompanhamento médico e tratamento.

O material sanguíneo coletado será devidamente armazenado no Núcleo de Pesquisas em Oncologia, para posterior análise relacionada exclusivamente ao presente estudo. Após a análise, o material restante será devidamente descartado, conforme normas vigentes de órgãos técnicos competentes, respeitando-se a confidencialidade dos voluntários da pesquisa. O seu consentimento para a guarda e utilização deste material pode ser retirado a qualquer momento, sem nenhum prejuízo ao seu tratamento no hospital.

IX - ACOMPANHAMENTO, ASSISTÊNCIA E RESPONSÁVEIS

Durante a pesquisa, caso ocorra algum dano referente à mesma, você receberá assistência integral e imediata, de forma gratuita, pelo período necessário, por parte dos pesquisadores.

O pesquisador e a equipe envolvida na pesquisa se comprometem a dar informação atualizadas ao longo do estudo, caso este seja o seu desejo.

TELEFONES PARA CONTATO EM CASO DE INTERCORRÊNCIAS CLÍNICAS, REAÇÕES ADVERSAS OU QUALQUER DÚVIDA SOBRE O ESTUDO:

TEREZA CRISTINA DE BRITO AZEVEDO (MÉDICA HEMATOLOGISTA) - 91 98817 2321
KARLA BEATRIZ CARDIAS CEREJA PANTOJA (BIOMÉDICA) - 91 98129 5453
MARIANNE RODRIGUES FERNANDES (BIOMÉDICA) - 91 99123 4727
NEY PEREIRA CARNEIRO DOS SANTOS (BIÓLOGO) - 91 99113 9221

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X. OBSERVAÇÕES COMPLEMENTARES:

Esta pesquisa poderá ser interrompida durante a sua realização e após o aval do CEP da instituição, em casos onde: a) O pesquisador responsável decida que algum motivo ou situação possa pôr em risco a segurança do participante ou; b) O CEP julgue que o estudo esteja sendo conduzido de maneira eticamente inaceitável.

Você tem garantida a liberdade de retirada de consentimento a qualquer momento e deixar de participar do estudo, sem qualquer penalização ou prejuízo à continuidade da sua assistência na instituição.

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XIII. QUEM DEVO CONTATAR EM CASO DE DÚVIDAS:
Pesquisador Responsável: DRA. TEREZA CRISTINA DE BRITO AZEVEDO – 91 98817 2321

Se o pesquisador responsável não fornecer as informações/ esclarecimentos suficientes, por favor, entre em contato com o **Comitê de Ética em Pesquisa em Seres Humanos do Núcleo de Pesquisa em Oncologia, de segunda-feira à sexta-feira das 9 horas às 15 horas, situado a Rua dos Mundurucus, 4487, nas dependências do Hospital Universitário João de Barros Barreto, prédio da UNACON, andar superior, e-mail cep.npo@gmail.com, telefone 3201-6778.**

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Este documento será elaborado em 2 (duas) vias. O (a) senhor (a) receberá uma delas e a outra será arquivada pelo pesquisador em seu arquivo.

Eu, ________________________________________________________________ declaro ter lido, compreendido e discutido o conteúdo do presente Termo de Consentimento e concordo em participar desse estudo de forma livre e esclarecida autorizando os procedimentos acima relacionados:

| ____________________________________ | ____________________________________ |
|---|---|
| Assinatura do participante | Assinatura do responsável pela pesquisa<br>(Dra. Tereza Cristina de Brito Azevedo) |
| ______/_______/_______ | ______/_______/_______<br>Data |