Title: Targeted therapy for CML patients on lmatinib: Role of novel variants of Human Organic Cationic Transporter 1 (hOCT1)

Abstract: SLC22A1 is the gene responsible for encoding Human Organic Cationic Transporter 1 (hOCT1), which is among a group of three similar transporters facilitating the uptake of numerous organic cations from the bloodstream into epithelial cells.hOCT1 shows a tendency towards several natural substances like dopamine, along with some medications like metformin, cimetidine, imatinib, etc.The expression of the hOCT1 gene is a reliable indicator of treatment response and clinical outcomes in patients before starting therapy. Furthermore, the expression of the hOCT1 gene has been known to influence the efficacy of imatinib therapy, with higher expression levels correlating with improved hematological and molecular responses.This chapter proposes that evaluating the expression levels of the hOCT1 gene can be a useful method for predicting the effectiveness of imatinib treatment in recently diagnosed chronic myeloid leukemia (CML) patients. Understanding the expression patterns and polymorphisms of the hOCT1 gene could thus inform the design of personalized therapeutic strategies tailored to individual patients at diagnosis. This knowledge could potentially optimize treatment outcomes and enhance the overall management of CML patients undergoing imatinib therapy.

Introduction: CML is a form of cancer that arises from abnormal growth of blood cells starting from hematopoietic stem cells. The development of CML is associated with a specific genetic anomaly called the translocation of chromosomes 9 and 22, also known as t(9;22) (q34;q11).This genetic event gives rise to the Philadelphia (Ph) chromosome, characterized by a shortened chromosome 22. The consequence of this translocation is the formation of a fusion oncogene called BCR-ABL. The BCR-ABL oncogene produces a hybrid protein, the 210 kDBcr-Abl

protein, comprised of an active Abl tyrosine kinase domain. This fusion protein plays a central role in the pathogenesis of CML, driving the uncontrolled proliferation and survival of leukemic cells(1). CML demonstrates a slight male predominance, with males being affected more commonly than females. Estimates suggest that the male-to-female ratio in CML incidence ranges from 1.3 to.4 to 1. This gender difference in prevalence underscores potential underlying biological or environmental factors contributing to the development of CML(2).The majority of patients are initially diagnosed during the chronic phase (CP). However, due to genetic instability, the disease can progress to a less defined and unstable accelerated phase (AP), and eventually the more severe blastic crisis phase (BP) as time goes on. This progression leads to increased resistance to treatment. The discovery of the BCR-ABL oncogene and its associated protein paved the way for the development of targeted small-molecule drugs that aim to disrupt the activation of the BCR-ABL tyrosine kinase. Imatinib was the first tyrosine kinase inhibitor (TKI) to be used and is generally well-tolerated, targeting the tyrosine kinase function of BCR-ABL. (4).TKIs resistance is a process involving Bcr-Abl dependent and independent resistance mechanisms. BCR-ABL independent mechanisms include non-compliance, poor intestinal absorption, drug interactions, altered levels of transporters, clonal evolution, SRC over-expression, etc. BCR-ABL-dependent mechanisms include mutations in the ABL-kinase domain.(3)

Soverini *et al* reported Researchers examined the occurrence of mutations in the BCR-ABL kinase domain based on the stage of disease at initial diagnosis. They discovered that 52% of patients with accelerated phase (AP), 75% with blast phase (BP), and 27% with chronic phase (CP) CML had mutations. This suggests that the frequency of mutations may contribute to resistance to imatinib and the progression from CP to BP. The primary biological consequence of

these mutations is that they render imatinib ineffective in inhibiting BCR-ABL kinase activity by preventing the protein from assuming an inactive state. Additionally, these mutations can disrupt BCR-ABL function, leading to cell death without detection, followed by the restoration of BCR-ABL function and the proliferation of mutated cells with reduced kinase activity (5).

Sokal and EUTOS scores are prognostic scores used for predicting response to therapy. Factors such as the patient's age, spleen size, percentage of certain blood cells, and platelet count are considered when assessing the risk level. Patients are then classified into high-risk or low-risk groups based on these criteria.

The hOCT 1 as pharmacogenetic: The human organic cation transporter 1 (hOCT1), also known as SLC22A1, is integral to the hepatic uptake of structurally diversified endogenous and exogenous organic cations, influencing both metabolism and drug pharmacokinetics. hOCT1 has been implicated in the therapeutic dynamics of many drugs, making interactions with hOCT1 a key consideration in novel drug development and drug–drug interactions. hOCT1 architecture conforms to the canonical two-fold pseudo-symmetry typical of the MFS transporters [14]. The protein can be divided into several different regions, with the N-terminal domain (NTD) comprising transmembrane helices 1–6 (TMs 1–6), and the C-terminal domain (CTD), consisting of transmembrane segments 7–12 (TMs 7-12). An extracellular domain (ECD) is delineated by a loop encompassing residues 44–142, situated between TM1 and TM2. On the intracellular side, helices form the intracellular helix (ICH) domain. Across all four characterized states — outward open, outward occluded, inward occluded, and inward open — the electron density maps of the transmembrane helices are resolved well enough to enable accurate model building. The predictive modelling of Alpha Fold2 further enhances this clarity (6). hOCT1 functions to transport a variety of diverse organic cations. Previous studies have established that genetic

polymorphisms influencing OCT1 activity can modulate the pharmacokinetics of drugs that are organic cations, affecting therapeutic efficacy and the likelihood of potential adverse effects(7). SLC22A1 is responsible for coding the Organic Cationic Transporter 1 (OCT1), among three closely related cationic transporters that facilitate the transfer of numerous organic cations from the bloodstream into epithelial cells. This transporter exhibits selectivity for various endogenous compounds and cationic medications. Imatinib has emerged as the primary treatment option for CML due to its notable effectiveness, minimal toxicity, and ability to sustain lasting hematological and cytogenetic responses. (8) Despite the initial success of imatinib in treating CML, challenges such as disease persistence and relapse have been reported, stemming from various mechanisms. While imatinib has shown high rates of hematologic and cytogenetic responses, around 25% of patients receiving imatinib as a monotherapy exhibit primary resistance or develop drug resistance, primarily due to mutations in the BCR-ABL kinase domain that hinder imatinib's binding ability.

Role of Imatinib drug and CML

Imatinib, a medication that inhibits tyrosine kinase, is now considered the gold standard for treating chronic phase CML. The majority of patients experience a complete cytogenetic response (CCR) with about half achieving a major molecular response (MMR) after three years of treatment. However, there are still some patients who do not reach CCR and some who lose their initial response. It is estimated that around 30%-40% of patients develop resistance or intolerance to imatinib. Various factors can contribute to this, such as mutations in the kinase domain and amplification of the BCR-ABL gene. The expression and activity level of hOCT1 have been identified as crucial factors in determining the response to imatinib, with low expression or activity indicating resistance to the medication. Studies have primarily focused on

imatinib uptake in the presence or absence of inhibitors to assess hOCT1 function and levels. However, there is limited data on how single nucleotide polymorphisms (SNPs) can affect the function of hOCT1 in CML. (9)

Recent studies have demonstrated patients with low expression or activity of hOCT1 had a lower probability of achieving a cytogenetic or molecular remission to CML. Improved progression-free and overall survival were also observed in patients with higher hOCT1 expression (10).In a study by Giannoudis *et al.*, the effect of polymorphisms rs628031 (Met408Val) and rs35191146 (420Del) on imatinib uptake and clinical efficacy was investigated (9). In CML cell lines transfected with the M420del and/or rs628031 (M408V), M420del significantly decreased imatinib uptake. Several papers find the uptake of Imatinib via hOCT1 controversial. For example, through transport and inhibition studies, Ann Nies and colleagues showed that overexpression of functional hOCT1 did not lead to increased accumulation of Imatinib. They go on to conclude that cellular uptake of imatinib is independent of hOCT1 and therefore hOCT1 is not a valid biomarker for imatinib resistance (11). In a separate study, the hOCT1 M420 deletion (rs35191146) was linked to the clinical outcome of imatinib-treated CML. Patients with this polymorphism demonstrated an increased probability of imatinib treatment failure. In a separate study, patients carrying the GG genotype for SNP rs683369 had a higher risk of losing response or treatment failure to imatinib therapy in CML patients (12).

hOCT1 gene expression:

Research on the activity of hOCT1 has mainly been centred around the absorption of imatinib, both with and without inhibitors. Limited information is available on how single nucleotide polymorphisms (SNPs) can impact the functionality of hOCT1 in CML. hOCT1 contains various

nonsynonymous SNPs that can influence the transportation of hOCT1 substrates. Numerous studies have explored the link between hOCT1 SNPs and the patient's response to imatinib, yielding conflicting findings.

Studies determined that variations in the hOCT1 gene known as M420del and M408V can impact the absorption of imatinib and influence the effectiveness of treatment in patients with CML. These specific genetic mutations, M420del and M408V (also known as rs628031), were identified with a frequency of around 18.5% and 59.8% in individuals of European-American descent, as reported by the National Center for Biotechnology Information (NCBI). According to their findings, M420del mutation has been linked to resistance to imatinib therapy. This was further validated through a study on imatinib absorption, revealing that the presence of M408V mutation could potentially reverse this resistance. (9) Wang L et al**,** reported that expression of hOCT1 is an important clinical determinant of the response to imatinib in CML. They found that imatinib uptake into a CML cell line with high hOCT1 expression was greater than those with modest or low expression (13).

Following previous studies, it can be concluded that hOCT1 gene expression alters the response to imatinib and high gene expression is associated with a better hematological and molecular response as compared to low gene expression. hOCT1 is an influx transporter that causes increased entry of imatinib into the cells. This could be the possible underlying mechanism by which response is altered.

Conclusion: Given these findings, it can be concluded that hOCT1 serves as a reliable biomarker for predicting response to imatinib therapy. Additionally, the SLC22A1 gene, which encodes

OCT1, plays a crucial role in the uptake of many commonly prescribed medications in the liver. Certain genetic variations in SLC22A1 may have important implications for clinical outcomes.

References:

1. Abdulmawjood, B.; Costa, B.; Roma-Rodrigues, C.; Baptista, P.V.; Fernandes, A.R. Genetic Biomarkers in Chronic Myeloid Leukemia: What Have We Learned So Far? *Natl. Libr. Med.* **2021**, *22*, 12516. [**Google Scholar**] [**CrossRef**]
2. Larson RA, Hochhaus A, Hughes TP, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive (Ph) chronic myeloid leukemia in chronic phase (CML-CP): ENESTnd 3-year follow-up. Leukemia. 2012; 26(10):2197-2203
3. Haiat S, Decleves X, Mittaine B, et al. Determination of imatinib plasma levels by high performance liquid chromatography (HPLC): evaluation of pharmacokinetic variability and haematological consequences. Blood 2006;108:1375a.
4. Gardner ER, Burger H, van Schaik RH, et al. Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. Clin PharmacolTher2006;80:192 ^ 201.
5. Soverini S, Bavaro L, De Benedittis C, et al. Prospective assessment of NGS-detectable mutations in CML patients with nonoptimal response: the NEXT-in-CML study [published correction appears in Blood. 2022 Mar 10;139(10):1601. doi: 10.1182/blood.2022015379]. *Blood.* 2020;135(8):534-541.
6. Jumper, J. et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021)
7. Kolz, C., Schaeffeler, E., Schwab, M. & Nies, A. T. Genetic and epigenetic regulation of organic cation transporters. *Handb. Exp. Pharmacol.* **266**, 81–100 (2021).

8. Polillo M, Galimberti S, Baratè C, Petrini M, Danesi R, Di Paolo A. Pharmacogenetics of BCR/ABL Inhibitors in Chronic Myeloid Leukemia. *International Journal of Molecular Sciences*. 2015; 16(9):22811-22829. https://doi.org/10.3390/ijms160922811

9. Giannoudis A, Wang L, Jorgensen AL, et al. The hOCT1 SNPs M420del and M408V alter imatinib uptake and M420del modifies clinical outcome in imatinib-treated chronic myeloid leukemia. *Blood*. 2013;121(4):628-637. doi:10.1182/blood-2012-01-405035

10. Marta Gromicho, Joana Dinis, Marta Magalhães, Alexandra R. et al. (2011) Development of imatinib and dasatinib resistance: dynamics of expression of drug transporters ABCB1, ABCC1, ABCG2, MVP, and SLC22A1. *Leukemia& Lymphoma* 52:10, pages 1980-1990.

11. Anne T. Nies1 , Elke Schaeffeler1 , Heiko van der Kuip1 , Ingolf Cascorbi4 , Oliver Bruhn4 . Cellular Uptake of Imatinib into Leukemic Cells Is Independent of Human Organic Cation Transporter 1 (OCT1), Clin Cancer Res; 20(4) February 15, 2014

12. Dong Hwan (Dennis) Kim,1,5 Lakshmi Sriharsha,1Wei Xu,2 Suzanne Kamel-Reid,3. Clinical Relevance of a Pharmacogenetic Approach Using Multiple Candidate Genes to Predict Response and Resistance to ImatinibTherapy in Chronic Myeloid Leukemia, Clin Cancer Res 2009;15(14) July 15, 2009

13. Wang W, Cortes JE, Tang G, et al. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. *Blood*. 2016;127(22):2742-2750. doi:10.1182/blood-2016-01-690230

14. Zhang S, Zhu A, Kong F, Chen J, Lan B, He G, Gao K, Cheng L, Sun X, Yan C, Chen L. Structural insights into human organic cation transporter 1 transport and inhibition. Cell Discovery. 2024 Mar 15;10(1):30.