SYSTEMIC MASTOCYTOSIS ASSOCIATED WITH THE C-KIT D816V MUTATION – IMPORTANCE OF MOLECULAR DIAGNOSIS AND THERAPEUTIC IMPLICATIONS. CASE REPORT

Adrian P. Trifa¹, Mariana Patiu², Andrei Cucuianu², Delia Dima², Mariela S. Militaru¹, Ioan V. Pop¹, Radu A. Popp¹

REZUMAT
Mastocitoză sistemică reprezintă o afecțiune hematologică malignă rară, caracterizată printr-o proliferare anormală a mastocitelor. Majoritatea pacienților sunt pozitivi pentru mutația somatică c-KIT D816V, care duce la activarea continuă a tirozin-kinazei KIT. Prezentăm un pacient de sex masculin în vârstă de 44 ani, cu o formă lent progresivă de mastocitoză sistemică. Am testat mutația c-KIT D816V prin tehnica AS-PCR (Allele Specific – Polymerase Chain Reaction) și am descoperit că pacientul este pozitiv pentru aceasta. A fost inițiat tratamentul cu imatinib mesylate, un inhibitor de tirozin-kinaze, dar acesta a fost oprit după 5 săptămâni, deoarece nu a fost consemnată nicio ameliorare în plan clinic. Testarea moleculară pentru mutația c-KIT D816V este obligatorie în mastocitoză sistemică, deoarece această mutație face parte din criteriile de diagnostic ale acestei afecțiuni. De asemenea, demonstrarea acestei mutații poate ghida atitudinea terapeutică, din moment ce mutația c-KIT D816V este asociată cu rezistență totală sau parțială la tratamentul cu inhibitori de tirozin-kinaze.

Cuvinte cheie: mastocitoză sistemică, mutația c-KIT D816V, inhibitori de tirozin-kinaze

ABSTRACT
SM (systemic mastocytosis) is a rare haematological malignancy, due to an abnormal proliferation of the mast cells; most of the patients are positive for the somatic c-KIT D816V mutation, which leads to continuous activation of the KIT tyrosine-kinase. We report on a 44 year old male patient with a slowly progressive form of SM. We investigated the c-KIT D816V mutation by AS-PCR (Allele Specific – Polymerase Chain Reaction) and found that the patient was positive for this mutation. A regimen with the tyrosine-kinases inhibitor imatinib mesylate was started, which was discontinued after 5 weeks due to lack of improvement in the patient’s clinical status. Testing for c-KIT D816V is mandatory in SM patients, since it represents a diagnostic criterion. Also, the c-KIT mutational status can guide the treatment, since the SM patients positive for c-KIT D816V are expected to be fully or partially resistant to tyrosine-kinases inhibitors.

Key Words: systemic mastocytosis, c-KIT D816V mutation, tyrosine-kinases inhibitors

INTRODUCTION
Mast cell disease (MCD) represents a rather heterogeneous group of usually malignant diseases in which the prominent feature is the excessive clonal proliferation of mast cells and their accumulation in one or more tissues.

¹ Department of Medical Genetics, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, ² Department of Haematology, Ion Chiricuta Cancer Institute, Cluj-Napoca

Correspondence to:
Adrian P. Trifa, Department of Medical Genetics, Iuliu Hatieganu University of Medicine and Pharmacy, 6 Pasteur Str., 400349 Cluj-Napoca, Romania, Tel. +40-750-774406, E-mail: adi_trifa@yahoo.co.uk

systemic mastocytosis and GISTs (gastrointestinal stromal tumors).

Under normal circumstances, the activated \( c-KIT \) is crucial for mast cell proliferation, maturation and survival.\(^5\) Somatic activating mutations in the \( c-KIT \) gene lead to continuous signaling in the KIT-mediated pathway and thus to abnormal proliferation of the mast cells.\(^4\) More than 90\% of the SM patients harbour somatic mutations within the \( c-KIT \) gene. The first \( c-KIT \) mutation to be described was a substitution of valine for aspartate D816V, resulting from a A-to-T transversion at position 2468, in exon 17 of the \( c-KIT \) gene \((c-KIT\text{c.2468A>T}).\(^6\)\) This mutation is by far the most common \( c-KIT \) gene alterations seen in SM patients, 80-90\% of the adult SM patients being positive for \( c-KIT \) D816V. Consequently, the presence of this mutation is considered a diagnostic criterion of SM according to the WHO classification.\(^3\,^4\)

**CASE REPORT**

A 44 year old male patient with a slowly progressive form of SM, diagnosed in 1983 and treated intermittently with corticosteroids, was referred to the Department of Haematology, Ion Chiricuta Cancer Institute, Cluj-Napoca, Romania. The patient had a generalized urticaria pigmentosum, submandibular and axillary lymphadenopathy, hepatomegaly (7 cm below the costal margin) and splenomegaly (4 cm below the costal margin). (Fig.1)

The blood counts revealed mild leucocytosis and the peripheral smear had an aspect suggestive of chronic myeloproliferative disease, with neutrophilia, eosinophilia, left shift and the presence of dysplastic, large platelets. The bone marrow biopsy revealed a 100\% cellularity, with 95\% infiltration by abnormal mast cells, intensely positive for tryptase and toluidin, displaying weak expression of KIT. (Fig. 2) The skin biopsy revealed dense groups of “fried-egg” like cells, corresponding to a massive dermal infiltration with mast cells.

Treatment was started with imatinib mesylate (Glivec\(^6\)) 400 mg/day and prednisone 20 mg/day. However, since there was no significant improvement, imatinib-mesylate was discontinued after 5 weeks and a maintenance dose of prednisone of 10 mg/day was given subsequently.

The \( c-KIT \) D816V mutation was assessed by an AS-PCR assay, as previously described by Schumacher et al, with minor modifications.\(^7\) Genomic DNA was obtained from peripheral leukocytes, using a commercially available procedure (Genomic DNA Purification Kit, Promega Ltd., MA, USA).

A fragment of 184 bp was obtained by PCR using two primers flanking the exon 17 of \( c-KIT \) gene; the 184 bp amplicon served as control for the PCR reaction; a second fragment, of 90 bp, was obtained using a forward primer which specifically hybridizes only with the allele containing the A-to-T transversion, which characterize the D816V mutation; the reverse primer was the same as for the control fragment. The 90 bp fragment is mutation-specific and is observed only in D816V-positive patients. As our patient DNA sample displayed both \( c-KIT \) fragments, of 184 bp and 90 bp, we concluded he was positive for \( c-KIT \) D816V mutation. (Fig. 3)

**DISCUSSION**

The \( c-KIT \) activating mutation status, especially the D816V mutation, represents a diagnostic criterion in systemic mastocytosis. Our patient was found to be positive for the D816V mutation, which is not surprisingly, because most of SM patients harbour this mutation.
Figure 3. Electrophoresis gel for the c-KIT D816V mutation. 1, 2, 3 – healthy individuals, displaying only the 184 bp fragment, which is gene specific; 4 – our SM patient, displaying both DNA fragments, the 90 bp fragment being specific to the c-KIT mutant allele; 5 – 100 bp DNA molecular weight marker.

Accordingly, screening for this mutation is mandatory in all patients suspected of SM. Several techniques for studying this mutation have been described, such as: direct DNA sequencing, dHPLC, PCR-RFLP, but while the first two are not affordable for all the laboratories, all of them have a quite low sensitivity, requiring at least 10% mutant cells from an analyzed sample, which is not always possible, especially when analyzing the cells from peripheral blood, from which usually only a small part harbour the mutation.\(^3\) However, the AS-PCR technique that we used to diagnose our patient has a much greater sensitivity, being able to detect less than 1% mutant cells in a given sample.\(^3\)

Imatinib-mesylate is a potent tyrosine-kinases inhibitor (TKI), active against several activating tyrosine-kinases mutations, including some of the c-KIT activating mutations. GISTs positive for c-KIT, which represent roughly 70% of the patients, were found to respond better to imatinib than c-KIT negative GISTs. Most of these cases harbour somatic mutations in exon 11 of the c-KIT gene.\(^7\) Resistance to imatinib occurs if these tumors acquire a second c-KIT mutation, either in the ATP-binding pocket (exon 13) or in the activation loop domain (exon 17). In fact, the c-KIT mutations within the activation loop domain, including D816V, are fully resistant to the inhibitory effect of imatinib, at least in vitro.\(^7\) Also, some clinical reports suggest that imatinib might not be very effective in the treatment of D816V-positive SM patients. Instead, second generation TKIs, such as dasatinib, are able to inhibit the D816V mutated c-KIT, at least in vitro. Dasatinib (Sprycel\(^\text{TM}\)) is a dual Src/Abl kinase inhibitor, which was shown in preclinical studies to inhibit both the growth of HMC-1.2 cells carrying the mutant c-KIT D816V and primary mast cells with c-KIT D816V mutation obtained from patients with SM.\(^8\) Even though dasatinib was beneficial in a proportion of SM patients, by improving disease-related symptoms, it didn't eliminate completely the disease in D816V-positive patients, suggesting that the benefit of dasatinib therapy in SM patients is only partial.\(^10\) Other agents, such as interferon-alpha and especially cladribine are effective in some SM patients with aggressive forms, but generally do not lead to long-lasting responses, while other TKIs, such as PKC 412 (midostaurine) are still in evaluation as a better choice in the treatment of SM.\(^11,12\)

In conclusion, testing for c-KIT D816V mutation has diagnostic and prognostic implications. While the SM patients who don't harbour this mutation do respond well to TKIs, the patients positive for D816V, like the patient we report on, are in general fully or partial resistant to TKIs. In general, these patients benefit only of symptomatic treatment, while specific therapeutic agents are still in evaluation.

REFERENCES