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Assessment of STRs Loci on Investigator Id-Plex Plus Kit for Forensics and Chimerism Monitoring in Morocco: A Case Series

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Abstract:- Allogeneic hematopoietic stem cell transplantation is a curative treatment for malignant hematologic diseases, leukemias, congenital and acquired non-malignant diseases of a patient (recipient) by replacing recipients cells by new one from a healthy donor. Monitoring of hematopoietic chimerism after allogenic transplantation is a useful tool for determining the engraftment of donor cells and predicting the risk of relapse of the original disease. Nowadays, diverses techniques are used and differ from laboratory to laboratory, which make data exchange and comparaison between them difficult. Amplification of short tandem repeats (PCR-STR) constitutes the gold standard method for chimerism quantification, although more sensitive PCR techniques have recently developed. This study was carried out on 30 allografted patients in whom the status of chimerism was analysed by the use of STRs included in Investigator Id-plex plus kit (Qiagen). This is conducted by taking samples of whole blood from these patients at different times in post-allograft from which we extract DNA and amplify STR markers and genotyped by capillary electrophoresis. The STR profiles have been generated for each sample including donor and recipient samples taken before the allogeneic transplant (J0). Profiles comparison obtained from the post-allograft samples with the genotypes of the donor and the pre-allograft recipient, permits to determine the status of chimerism in these different patients. This work was preceded by analyzing a set of 219 individual from Morocco with the objective to establish their usefulness for human identification. Allelic frequencies for the 15 short tandem repeat (STRs) loci were calculated. Results show that the 15 loci are highly polymorphic. The combined power of exclusion for the fifteen loci is 0.99999968 and the combined discrimination indice was 0.9999999999999999985. The combined matching probability for these loci reaches 1.59 10^{-18} which make these loci very useful for personal identification casework purposes in Morocco.

Keywords:- *Chimerism; STR; Morocco; Allogenic Transplanted Patients; Investigator IDPlex Plus; Informativity; Polymorphism, Forensic Parameters*

I. INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is curative treatment for malignant hematologic diseases, leukemias, congenital or acquired non-malignant diseases [1, 2]. It consists to the substitution of damaged hematopoietic cells of a recipient (patient) by a new one from a healthy donor. The coexistence of cells with different genetic origins (donor and recipient) in a patient after receiving a hematopoietic stem cell transplant (HSCT) is called "chimerism". It's defined by the percentage of cells of donor origin in the recipient patient, either in the blood or in the marrow [3-5]. Chimerism monitoring is actually a routine diagnostic tool at allogenic transplant centers and it is useful for evaluating the stability of lymphoid and myeloid donor engraftment to distinguish the success or failure of the transplant, to predict the possibility of a relapse, and to apply the opportune therapy by physicians [6-7]. Complete chimerism (CC) is characterized by a total replacement of the patient's hematopoiesis by that of the donor, and a mixed chimerism (MC) by the persistence of the two types of cells (patient and donor). An increase in the percentage of chimerism refers to an amplification in the percentage of cells obtained from the donor, with a MC approaching CC. Mixed chimerism can have two different meanings: either it is normal mature cells of the patient, having survived conditioning or having matured from residual hematopoietic stem cells from the recipient; or they are residual malignant cells that have survived the conditioning and may cause a relapse. MC can evolve to the loss of the graft or a relapse of the hemopathy [1,8]. Very early MC would generally be explained by the persistence of normal recipient cells, and not by the presence of malignant cells. Early analyses of recipient chimerism patterns increase the importance of predicting graft rejection as well as persistent disease or failure [9].

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Hematopoietic chimerism classification was based on chimerism analyses using quantitative and qualitative tests based on exploring genetic polymorphism for the identification of the recipient's and donor's cells [5, 10, 11]. In the past, several markers that have been successively used, such as the blood groups of the ABO system, or HLA antigens. These techniques were time-consuming, poorly informative, or studying a single lineage. They were generally uninformative in the event of a blood transfusion [12, 13]. Conventional cytogenetics has made it possible to develop a genetic approach, by detecting either the sex of the patient and the donor, or specific abnormalities of the disease (Philadelphia chromosome in CML). Because this technique is not very sensitive (5-10%), and not quantitative because of the culture; it has been replaced by the FISH (*Fluorescent in situ hybridization*) using fluorescent probes specific for sequences carried by the X and Y chromosomes allowing more precise quantification (sensitivity less than 1%)[14].

The use of molecular biology techniques has a good impact on the quality of chimerism analysis results. It started by the study of RFLP (*Restriction Fragment Length Polymorphism*) [15, 16]. This technique is semi-quantitative, insensitive (5-10%) and consumes a lot of biological material. The introduction of markers discovered in 1985 [17] commonly called "genetic fingerprints" based on PCR-VNTRs (*Variable Number Tandem Repeats*) (size between 10 to 50 bp) [11, 18] allowed the development of chimerism monitoring [19-22].

Later, VNTRs analyses have been replaced by studying "Microsatellite" sequences: STR (*Short Tandem Repeats*) by PCR which are smaller, comprising 2 to 7 bp repeat [23]. They are highly variable from one individual to another and distributed throughout the human genome and are therefore highly informative. They are tool used as genetic fingerprints in forensic medicine and for paternity testing as we can analyze many loci at once on small quantities of DNA, even degraded by multiplex PCR. Nowadays, the analysis of short tandem repeats (STRs) by polymerase chain reaction (PCR) combined to capillary electrophoresis is the most commonly used procedure for chimerism quantification which sensitivity is about 3–5%. In an allograft context, the small amounts of DNA required make it possible to obtain a result from the immediate post-allograft period, when the patient is still in aplasia, with hypocellular marrow [24].

The study of SNP (*Single Nucleotide Polymorphism*) by quantitative real-time PCR TaqMan® (ThermoFisher, USA) and Droplet digital PCR (ddPCR) [25] as well as small insertions and deletions polymorphisms, can be detected by allele-specific quantitative PCR [26, 27]. Quantification by these methods is therefore very precise in low percentages, currently usable routinely (10^{-5} to 10^{-3}) since their mutation rate is smaller than the mutation rate of STRs and may provide additional information in some cases where STRs assays present sensibility issues, with potential use in monitoring residual disease in a context of standard conditioning or attenuated conditioning, once CC is reached [13, 28]. The threshold from which a chimerism is considered complete depends on the sensitivity of the technique. Insensitive methods cannot differentiate a 90% donor chimerism from a 95% donor chimerism. The difficulty is to define a threshold that is significant from a medical point of view, but also quantifiable by the technique used. In general, a CC is considered if the percentage of cells derived from the donor is greater than 95% donor.

Chimerism analysis should be carried out in the routine with efficient techniques in terms of power of discrimination, cost and time, thus recommendations are focused in the use of STR markers as it's the most used and standardized techniques [29- 31]. The analysis of chimerism by STR–PCR usually is frequently carried out with commercial kits originally designed for forensic purposes, including a large number of STR, and cover diverse global human populations [32-35]. Although the frequency of alleles differs from one population to another [36]. The inclusion of loci with important differences in a power of discrimination (PD) across different populations is inefficient as some loci often become less informative for chimerism analysis in some populations [37]. Thus, some studies try to elucidate which are the most informative markers [38-40]. Few studies have been conducted to evaluate the informativeness for human identification of certain STR loci included in commercial kits: 8 autosomal loci of Promega Kits [41], 15 autosomal STRs loci included in Identifiler Kit [42 , 15 Y STRs included in Y-filer [43].

This study aims to assess either the genetic diversity of the 15 STR loci (CSF1PO, FGA, TH01, TPOX, vWA, Amelogenin D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11) included in the Investigator IDplex-plus kit (Qiagen, Hidden, Germany) to explore the potential of applying for human identification in a population sample and to assess their usefulness for chimerism after allogenic transplantation in Moroccan patients to define the most useful in the perspective to identify a minimal STR panel allowing easy, fast and cost-effective monitoring of chimerism in allogenic bone marrow transplantation.

II. MATERIAL AND METHODS

➢ *Population Analysis*

- *Samples and Methods*

For the generation of population data, 219 saliva samples on FTA cards were obtained from genetically unrelated individuals. All donors read and signed a written consent statement form in accordance of the Hensilki declaration. Direct genotyping analysis workflow was conducted without DNA extraction: a 0.2mm punches were deposited on a 96 well containing 150 µl of water, incubated for 10 min, and the water was discarded but 5µl left in the well. 10µl of PCR master mix for amplification of 15 STR loci included in the Investigator Idplex plus kit (Qiagen, Hiden, Germany) was added (following manufacture protocol). Amplification was conducted on Veriti thermocycler (Thermo Fisher Scientific) using manufacture protocol with slight modifications (27 cycles and expansion time 60° C for 45 min). The post-PCR mix was added to a

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96-well genotyping plate. The post-PCR mix consisted of Hi-di formamide (9.3 µL per well) and Internal Lane Standard BTO-500 (0.7µL per well). One microliter of each PCR product was added directly to the corresponding well, denatured for 3 min at 95 °C and cooled at 4 °C. Fragment separation was performed by capillary electrophoresis on an ABI3130XL Genetic Analyser (Thermo Fisher Scientific). Data collected were analyzed using the GeneMapper® ID-X v1.4 software (Applied Biosystems) for genotyping step.

- *Statistical Analysis of Data:*

GENEPOP software (Version 3.3) [44] was used to calculate the allele frequencies and to perform the exact test of Hardy-Weinberg [45]. The observed heterozygosity (Ho), expected heterozygosity (He), the power of discrimination (PD), the probability of match (PM), the Polymorphic Information Content (PIC) and the Power of Exclusion (PE) were calculated using CERVUS software [46].

➢ *Chimerism Analysis*:

Our series includes 30 patients suffering from malignant (acute leukemia: LLA MLA…) and non-malignant diseases (medular aplasia, immune deficiency..), with bone marrow transplantation subjected to chimerism analysis. This study was approved by the ethics committee of 20 Aout Hospital according to the declaration of Helsinki protocol and recipients and donors gave written informed consent before bone marrow transplantation. Blood samples from the recipient and the donor were taken before transplantation, so the genotypes of the donor and the recipient are known. Furthermore, recipient blood samples at various time points post-transplant (J30, 60, 90…..etc),. A total of 98 post-HSCT peripheral blood samples (2-5 ml) were collected since January 2019 (See Table 2 for details), 250-300 µl of blood were used for DNA extraction using the EZ1 Investigator DNA extraction kit following the manufacturer's instructions (Qiagen, Hiden, Germany) on EZ1 automate. Microsatellite amplification was conducted using 3-5 µl of DNA and amplification and genotyping were conducted as mentioned below. Genotypes were analyzed using the GeneMapper® ID-X v1.4 software (Applied Biosystems). As defined by UK NEQAS technical recommendations for chimerism analysis using STR-based techniques, only fully informative markers were selected [11,40]. Loci considered for chimerism calculation were those showing more than two alleles and showing recipient alleles outside of the stutter positions of the donor's peaks, usually referred to as 'type 5' [11]. A minimum of three informative STR markers were used to avoid misinterpretation in case of genetic alteration involving one locus or the increase of microsatellites mutations rate in hematological malignancy relapse. Markers showing stutter interference were excluded because interpretation is hence difficult.

Chimerism calculation was based on the fluorescence intensity of each allele designed as peak height ratio "PHR" that depends on PCR amplification yield based on the DNA concentration. The use of too much DNA induces the apparition of saturation and stutters that can influence the chimerism interpretation. Thus, DNA concentration used around 5-10 ng (and less DNA quantity should not be used to avoid the problem of stochastic amplification tending to provide less reliable STR- profiles (low copy number) [47]. Based on the analysis method parameters for GeneMapper®ID-X v1.4 software for forensic purposes, it's obvious that for reference samples, it's usual to increase the Pic Height Ratio (PHR) to avoid misinterpretation of stutters, on the contrary, casework samples have to be interpreted with lower PHR to permit mixture interpretation. The same reflection has to be followed in the case of chimerism interpretation. In case of mixed chimerism analysis, the pic height ratio = 50 RFU was very useful for some loci to detect chimerism than the use of 100-150 RFU which could be misinterpreted as complete chimerism status (Figure1).

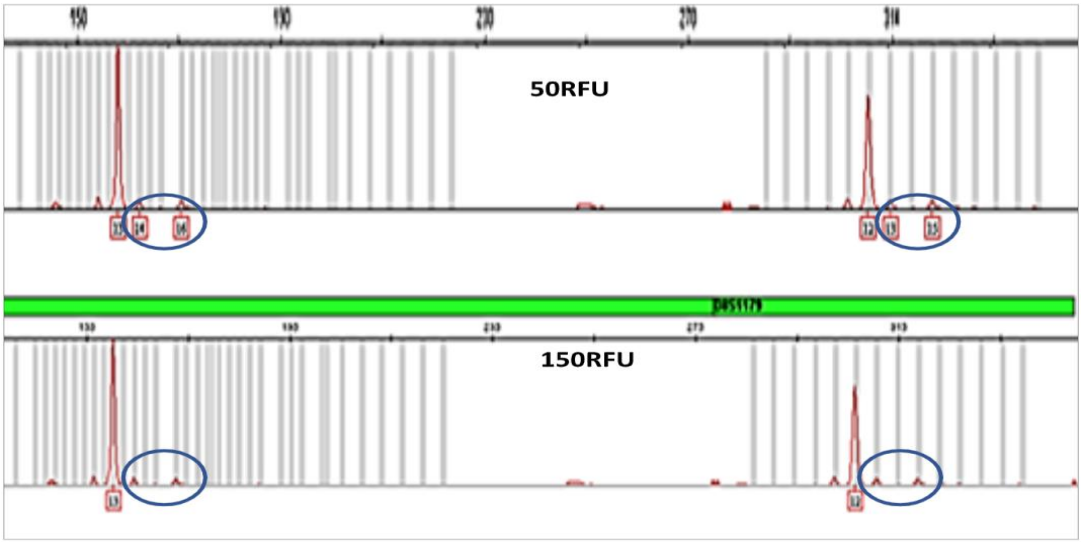

Fig 1 Difference in Allele Size Calling Depending on Pic Height Ratio (PHR) Parameters

The percent donor chimerism for each locus was estimated as the sum of donor allele areas divided by the sum of the areas of all alleles in a given locus (donor + receiver) [48]. For each sample, we estimated the percent donor chimerism (mean,) and the number of STRs microsatellites showing chimerism.

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III. RESULTS

➢ *Population Analysis:*

A total of 156 alleles were detected with corresponding allele frequencies ranging from 0.0023 to 0.4521 (see Table1). The most polymorphic STR marker was D19S433 with 18 alleles and the less polymorphic markers are (D3S1358, TPOX, D5SS818, D16S539) with 7 alleles. The Hardy-Weinberg equilibrium exact test using 2000 shuffling showed two departures from the equilibrium for vWA and D21S11 (a 5% significance level is taken, table 1). After employing a Bonferroni Correction for the number of loci analyzed, these observations are not likely to be significant. The observed heterozygosity varies from 0.612 for D21S11 to 0.8447 for D8S1179. The mean observed heterozygosity across all loci reach 80%. As expected, the number of alleles is correlated with polymorphic information content PIC. The power of discrimination (PD) varies from 0.906 (D16S539) to 0.972 (D18S51). The probability of exclusion varies from 0.470 (CSF1PO) to 0.765 (D21S11) and the combined power of exclusion of the 15 loci is 0.99999968. The combined matching probability for the 15 loci reached 1.59 10^{-18}, that make the Investigator IDplex-plus kit (Qiagen, Hidden, Germany) a highly polymorphic tool for the human identity in the Moroccan population.

➢ *Chimerism Analysis:*

Our cohort includes 30 patients, with a sex ratio M/F of 1.14 and ages between 4 and 63 years old, presenting different pathologies (see Table 2). The myeloid acute leukemia (AML) and medullar aplasia (30% each) are the two major cause of allogenic hematopoietic stem cell transplantation (ASCT) followed by acute lymphoid leukemia (ALL) (20%) (Figure2). In fact, malignant diseases represent the major cause of ASCT than non-malignant diseases (67% *vs* 33 %).

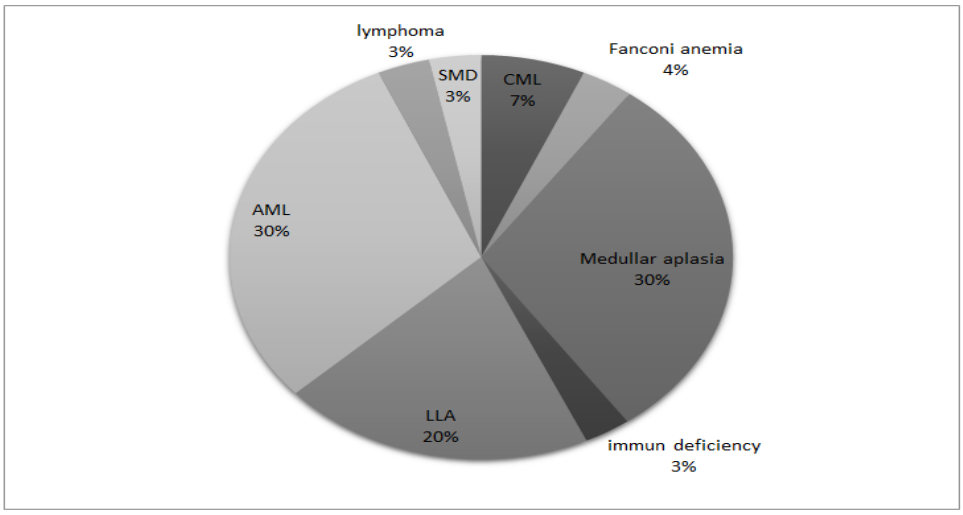

Fig 2 Distribution of Patients Based on the Type of Pathologies

Chimerism is a dynamic process with proportions variation of donor cells over time, the use of STRs gives reproducible results and can assess samples at different time points to conduct longitudinal studies and produce an inclusive chimerism analysis report (Table 2). As shown in Figure 3, the informativity of the STR markers varied widely, between 63.3% and 20%. The most informative STR loci were D8S1179, D21S11, FGA, D18S51, D2S1338 and TPOX which individually allowed direct detection of chimerism in more than 50% of the cases. In contrast, the least informative markers were D13S317 and CSF1PO. As expected, informativity and heterozygosity tended to be correlated, although imperfectly (TPOX).

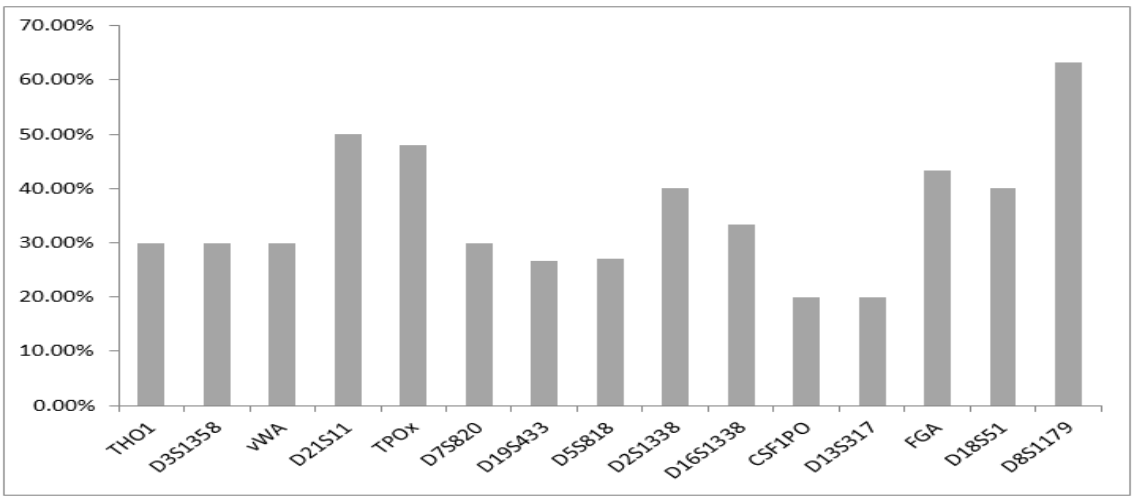

Fig 3 Loci Informativity for Chimerism Monitoring

Chimerism status has been established for the patients included in this study as complete chimerism (CC) or mixed chimerism (MC) or absence of chimerism. We identified 37 samples showing mixed donor–recipient chimerism (MC) while 47 samples showed complete chimerism (CC). For 14 samples a recipient profile has been detected as a consequence of the no engraftment success. Relationship between the nature of the disease and the success/or no of engraftment was not established.

In patients with non-malignant hemopathies, four patients showed complete chimerism status (CC), however mixed chimerism status (MC) was observed in five patients and two patients showed transplant rejection. On the other hand, patients suffering from malignant hemopathies showed complete chimerism and mixed chimerism in nine and four patients respectively. Three patients showed transplant rejection and three patients showed complete chimerism at the beginning followed by mixed chimerism status subsequent to D60 (1 patient) or D90 (2 patients). From our cohort of 30 patients, 22 patients are still alive (until the redaction of this article) and 8 died as mentioned in Table 3 from wish only 3 persons with an acute leukemia disease die after relapse, all supporting a complete chimerism status until D90 and D180 respectively.

IV. DISCUSSION

Chimerism monitoring is based on STR profil comparaison after allogenic transplantation. The informativity of an STR in chimerism analysis could be defined as its capability to distinguish between the donor and the recipient cell components and implies that at least one allele should differ between the recipient from those of the donor [49]. The distinction between donor and recipient alleles in the post-transplant genotype is easy when both the pretransplant genotypes of the recipient and the donor are available to be compared with the post-transplant genotype. However, in routine medical practice, sometimes we lack one or two of those genotypes for comparison. In our case, the lack of donor/or recipient sample before transplantation impacted the interpretation of one case only. Furthermore, our STR evaluation criteria take into consideration either the potential influence of stutter peaks originated from polymerase slippage during PCR that appear one repeat unit smaller than the authentic allele and represent 4–11% of the major component height, so they can be misinterpreted as a true minor allele (39,50-51); and the peak height ratio imbalance. When the pre-transplanted and donor samples are available, the comparison of those genotypes with the post-transplant genotype is easy even they share alleles. But in case of lack of one genotype or the two genotypes, the interpretations became difficult. The use of polymorphic markers would help chimerism interpretation but will be specific to population genetic polymorphism [52]. The FGA, D18S51, D21338 and D21S11 STR markers have been reported before to be very useful for chimerism in Spanish population study [39].

The importance of chimerism study after ASCT is to predict negative events like graft rejection, disease relapse, and GVH disease in order to interfere with appropriate medical therapy. Chimerism, especially complete chimerism, has been associated with an increased risk of relapse in certain types of malignant diseases, such as leukemias and lymphomas. From our cohort only 3 persons with an acute leukemia disease die after relapse, all supporting a complete chimerism status until D90 and D180 respectively. This is due probably to the expansion of recipient leukemic cells surviving after conditioning therapy. The chimerism status evaluation in these cases didn't permit to detect of the high risk of relapse after CC, and should be monitored otherwise by conducting an STR-PCR chimerism analysis not on peripheral blood but from separated lineages blood cells (ex T- and NK-cell) or bone marrow sample to permit the detection of any transformation earlier before the relapse expression [9] or like detecting residual leukemia in the form of MRD [2] , especially in those still alive with complete remission after CC status. Only in this case, we can propose a very sensitive method like q-PCR to monitor chimerism as a secondary tool besides STR-PCR markers.

However, it is important to note that the risk of relapse in transplant recipients with mixed chimerism is generally lower than the risk of relapse in non-transplanted individuals with the same malignant disease. The variation of the degree of the MC may not be influenced by the disease but rather by other factors including its stage at the time of ASCT, the conditioning regimen, and the timing of the assay (time from sampling until treatment should be considered as critical parameter). Additionally, the use of immunosuppressive therapy and other treatments can help to control the risk of relapse in transplant recipients with chimerism.

Moreover, the correlation between MC and relapse is still a controversial issue but seems to be more likely correlated in acute leukemia (2 patients with MC showed disease relapse) [53-54] It is important to note that monitoring chimerism should be done in conjunction with other forms of monitoring, such as clinical examination, imaging studies, and laboratory tests, to identify potential signs of relapse as early as possible.

V. CONCLUSION

In conclusion, Investigator Id-plex plus kit offers either a highly polymorphic tool for the human identity in the field of forensics in the Moroccan population and a powerful and relatively low-cost molecular method for helping in practice medical patient care decisions for chimerism monitoring. The use of the markers included in the Investigator IDplex plus kit (Qiagen, Hidden, Germany) showed their usefulness and we can choose those most polymorphic in our population to reduce the cost of analysis. Increasing our sampling will give more insight about the power of STR for allogenic transplantation monitoring.

- *Conflicts of Interests:* Authors declare no conflict of interest.

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Table 1 Observed Allele Frequencies and Statistical Parameters for *Investigator Idplex plus* Kit Loci in Moroccan Population:

Alleles	**TH01**	**D3S1358**	**vWA**	**D21S11**	**TPOX**	**D7S820**	**D19S433**	**D5S818**	**D2S1338**	**D16S1338**	**CSF1PO**	**D13S317**	**FGA**	**D18S51**	**D8S1179**
5	0.0068	-	-	-	-	0.0023	-	-	-	-	-	-	-	-	-
6	0.1735	-	-	-	0.0180	-	-	-	-	-	-	-	-	-	-
6.3	-	-	-	-	-	0.0023	-	-	-	-	-	-	-	-	-
7	0.2329	-	-	-	0.0124	0.0046	-	-	-	-	0.0046	-	-	-	-
7.3	0.0023	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	0.1872	-	-	-	0.4521	0.1073	-	0.0502	-	0.0160	0.0228	0.0959	-	-	0.0068
9	0.2283	-	-	-	0.1849	0.1142	-	0.0320	-	0.1187	0.0166	0.0365	-	-	0.0046
9.3	0.1210	-	-	-	-	0.0114	-	-	-	-	-		-	-	-
10	0.0457	-	-	-	0.0869	0.3562	0.0023	0.0662	-	0.0731	0.3356	0.0388	-	0.0068	0.0639
11	0.0023	-	-	-	0.2192	0.2420	0.0160	0.2580	-	0.2900	0.2783	0.2854	-	0.0205	0.1142
11.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	0.0255	0.1438	0.1438	0.3721	-	0.2991	0.2988	0.3858	-	0.1396	0.1393
12.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	0.0046	0.0023	-	-	0.0160	0.2489	0.1986	-	0.1849	0.0365	0.1096	-	0.1396	0.1941
13.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	0.0434	0.1256	-	-	-	0.2511	0.0228	0.0023	0.0183	0.0068	0.0434	-	0.1426	0.2580
14.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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15	-	0.3457	0.1438	-	-	-	0.1096	-	-	-	-	0.0046	-	0.1220	0.1758
15.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	0.2795	0.2580	-	-	-	0.0388	-	0.0434	-	-	-	-	0.1461	0.0411
16.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	0.1973	0.2511	-	-	-	0.0137	-	0.3174	-	-	-	-	0.1540	0.0023
17.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	0.1233	0.1324	-	-	-	0.0023	-	0.0799	-	-	-	-	0.0525	-
18.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0046	-	-
19	-	0.0052	0.0616	-	-	-	-	-	0.1005	-	-	-	0.0457	0.0444	-
19.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	0.0183	-	-	-	0.0023	-	0.1575	-	-	-	0.1050	0.0068	-
21	-	-	0.0068	-	-	-	0.0023	-	0.0594	-	-	-	0.1895	0.0183	-
21.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0023	-	-
22	-	-	-	-	-	-	0.0114	-	0.0434	-	-	-	0.1872	0.0046	-
23	-	-	-	-	-	-	0.0274	-	0.0822	-	-	-	0.2032	0.0023	-
23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	0.0434	-	0.0776	-	-	-	0.1393	-	-
24.2	-	-	-	-	-	-	-	-	-	-	-	-	0.0023	-	-
25	-	-	-	-	-	-	0.0502	-	0.0274	-	-	-	0.0616	-	-
25.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	0.0320	-	0.0091	-	-	-	0.0457	-	-
27	-	-	-	0.0274	-	-	0.0023	-	-	-	-	-	0.0068	-	-
28	-	-	-	0.0959	-	-	0.0023	-	-	-	-	-	0.0046	-	-
28.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28.3	-	-	-	0.0776	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	0.1644	-	-	-	-	-	-	-	-	0.0023	-	-
29.2	-	-	-	0.0023	-	-	-	-	-	-	-	-	-	-	-
29.3	-	-	-	0.1370	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	0.1461	-	-	-	-	-	-	-	-	-	-	-
30.2	-	-	-	0.0068	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	0.0251	-	-	-	-	-	-	-	-	-	-	-
31.2	-	-	-	0.1164	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	0.0114	-	-	-	-	-	-	-	-	-	-	-

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32.2	-	-	-	0.1301	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	0.0023	-	-	-	-	-	-	-	-	-	-	-
33.2	-	-	-	0.0411	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34.2	-	-	-	0.0091	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	0.0068	-	-	-	-	-	-	-	-	-	-	-
N° allele	**9**	**7**	**9**	**16**	**7**	**10**	**18**	**7**	**12**	**7**	**8**	**8**	**14**	**14**	**10**
HO	0.7945	0.731	0.740	0.612	0.676	0.7489	0.8265	0.7763	0.7763	0.7808	0.6986	0.7260	0.8356	0.8356	0.8447
HE	0.8136	0.750	0.814	0.886	0.707	0.7707	0.8359	0.7488	0.8390	0.7740	0.7224	0.7454	0.8512	0.8512	0.8285
P	0.2382	0.3665	0.0020	0.0009	0.1521	0.2829	0.9162	0.3160	0.4910	0.1647	0.9035	0.3568	0.7015	0.7015	0.1664
PD	0,937	0,895	0,939	0,975	0,871	0,913	0,953	0,897	0,958	0,913	0,870	0,898	0,959	0,972	0,947
PE	0,623	0,518	0,629	0,765	0,475	0,562	0,677	0,526	0,691	0,560	0,470	0,530	0,698	0,749	0,655
PIC	0.784	0.706	0.786	0.872	0.663	0.736	0.815	0.708	0.822	0.737	0.669	0.707	0.831	0.863	0.804

- *HO: observed heterozygosity; HE: expected heterosygosity; P: Hardy-Weinberg equilibrium exact test; PD: power of discrimination; PE: power of exclusion; PIC: polymorphism information content*

Table 2 Summary of the Patients Data and Chimerism Status Evaluated using Investigator IDplex Plus STR Markers

N°	AGER	sexeR	sexeD	pathology	J30	J60	J90	J180	J240	J365	J398	J600	775	J900	J1000	status	transplant status
P1	63	F	F	CML	CC	MC 73 73%	MC 77%	x	x	x	MC, 67%	MC 89%	x	x	x	Alive	CR
P2	35	F	M	AML	CC	CC	CC	CC	x	x	x	x	x	x	x	Alive	Relapse
P3	5	M	M	AML	x	x	R	R	x	x	x	x	x	x	x	Alive	transplant rejection
P4	56	F	F	AML	CC	CC	MC 91,5 %	x	x	x	x	x	x	x	x	Dead	CR
P5	62	F	M	AML	MC 90,5 %	MC 50%	MC 67,4 %	MC 72,2 %	x	x	x	x	x	x	x	Alive	CR, deceased after COVID-19
P6	44	M	M	AML	MC 92%	MC 72,6 %	x	x	x	x	x	x	x	x	x	Dead	Relapse
P7	35	F	F	AML	MC 84%	MC 88%	MC 88%	MC 92%	MC 93%	x	x	x	x	x	x	Dead	CR
P8	44	F	M	AML	CC	CC	CC	x	x	x	x	x	x	x	x	Alive	Relapse
P9	25	M	F	AML	MC 92%	MC 80,7 %	MC 78,8 %	MC 85,9 %	x	x	x	x	x	x	x	Alive	Relapse
P10	45	M	M	AML	CC	CC	CC	x	x	x	CC	x	x	x	x	Alive	CR

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P11	22	M	M	AML	CC	CC	CC	CC	X	X	X	x	x	x	x	Alive	CR
P12	47	F	M	LLA-B	CC	x	x	x	x	x	x	x	x	x	x	Alive	RAS
P13	22	M	M	LLA-T	CC	CC	CC	x	x	x	x	x	x	x	x	Alive	Relapse
P14	37	M	M	LLA-T	R	R	R	R	X	X	x	x	x	x	x		CR
P15	39	F	M	LLA-T	x	CC	CC	CC	x	x	x	x	x	x	x	Alive	Relapse
P16	35	F	M	LLA-T	x	x	x	x	x	CC	x	x	x	x	CC	Dead	CR
P17	18	M	M	LLA-T	CC	CC	CC	CC	x	x	x	CC	x	x	x	Dead	CR
P18	34	M	F	SMD	R	R	x	x	x	x	x	x	x	x	x	Dead	post transplant infection
P19	17	M	M	Fanconi anemia	x	x	MC 87%	x	x	x	x	x	x	x	x	Alive	CR
P20	25	F	M	Hodgkinien lymphoma	CC	CC	MC 95%	x	x	x	x	x	x	x	x	Alive	CR, death due a transplant toxicity
P21	4	M	F	immun deficiency	x	R	R	x	x	x	x	x	R	R	x	Alive	CR, autologos reconstitution
P22	25	F	F	Medullary aplasia	MC 81,5 %	MC 92,2 %	MC 91%	x	x	x	x	x	x	x	x	Alive	CR
P23	19	M	F	Medullary aplasia	MC 93%	MC 75,4 %	MC 85,9 %	MC 76,9 %	x	MC 86%	x	x	x	x	x	Alive	CR
P24	20	F	F	Medullary aplasia	CC	CC	CC	CC	x	x	x	CC	x	CC	x	Alive	CR
P25	21	F	M	Medullary aplasia	x	CC	x	CC	x	x	x	x	x	x	x	Alive	CR
P26	28	M	F	Medullary aplasia	MC 75%	MC 61%	MC 64%	x	X	X	X	x	x	x	x	Alive	CR
P27	20	M	F	Medullary aplasia	x	R	R	x	x	x	x	x	x	x	x	Alive	Relapse after 2 ASCT
P28	29	F	M	Medullary aplasia	CC	CC	x	x	x	x	x	x	x	x	x	Alive	CR
P29	34	M	M	Medullary aplasia	MC 96%	X	X	MC 80,3 %	X	X	X	x	x	x	x	Dead	CR
P30	17	M	F	Medullary aplasia	CC	CC	CC	CC	x	CC	x	x	x	x	x	Alive	CR

(R: recipient; CC: complete chimerism, MC: mixed chimerism; CR: complete remission)

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Table 3 Status of the patients at the end of this study and main causes of death.

Patients still alive: 22 patients	**Dead patients: 8**
• Relapse (3) • No engraftment (1) • Complete remission (17) or Autologous recovery (1)	• Relapse (3) • Non engraftment (1) • Post transplantation infection (1) • COVID-19 (1) • Digestive GVH disease (1) • Pulmonary fibrosis (grapht toxicity) (1)