Prima
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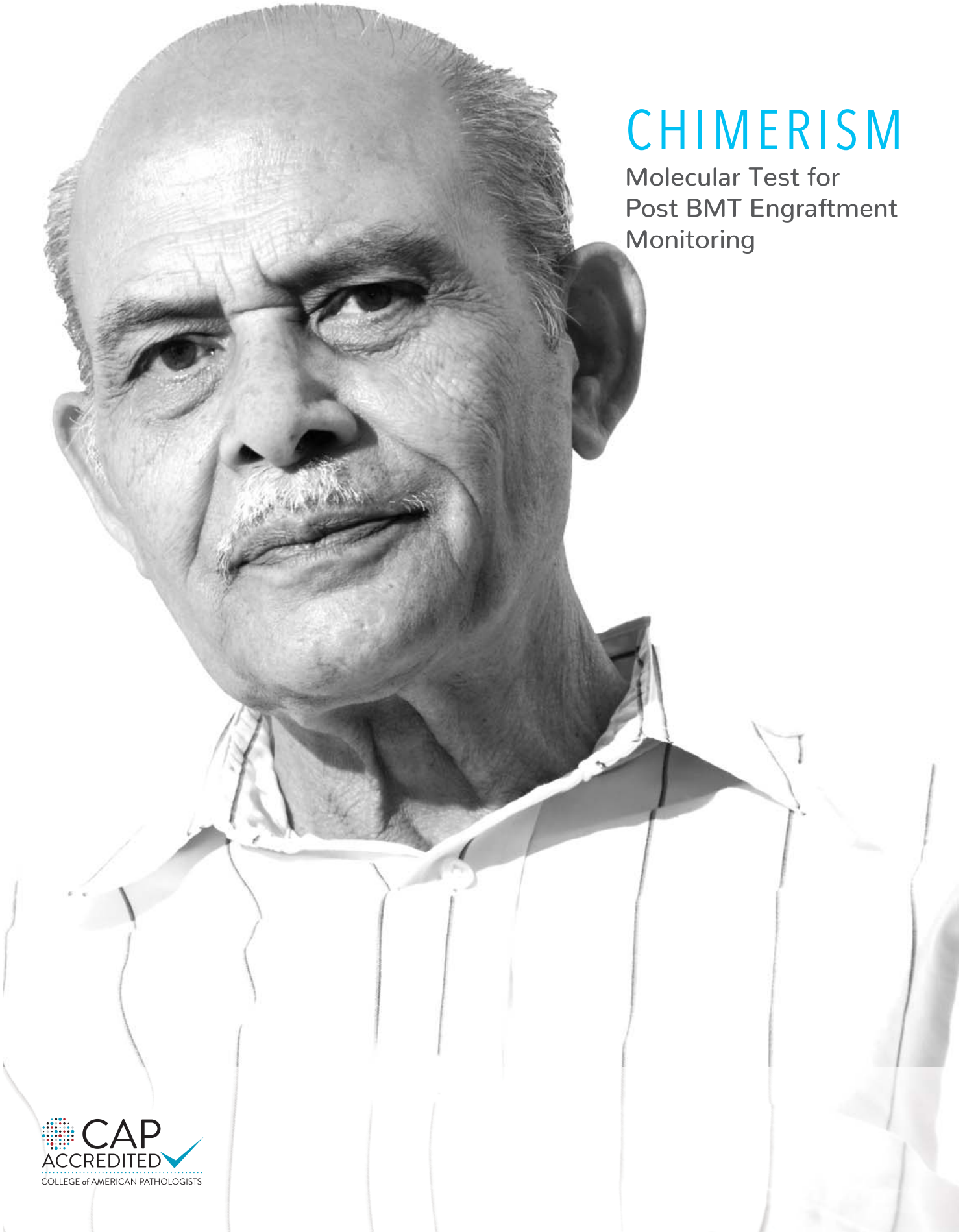
MEDGENOME



CHIMERISM

Molecular Test for Post BMT Engraftment Monitoring

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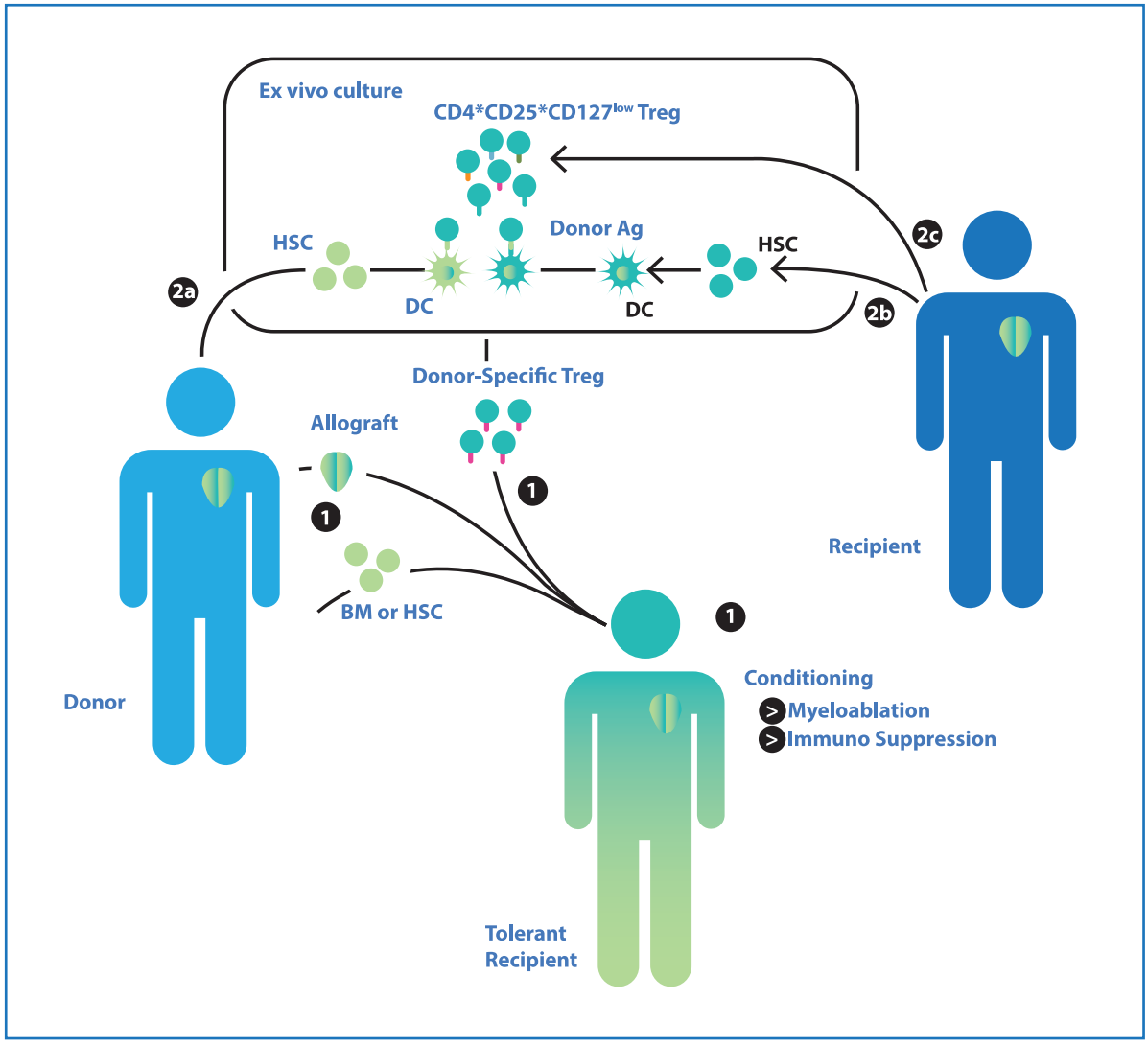
Post Bone Marrow Transplant Engraftment Monitoring

Post Bone Marrow Transplant Engraftment Monitoring

Introduction

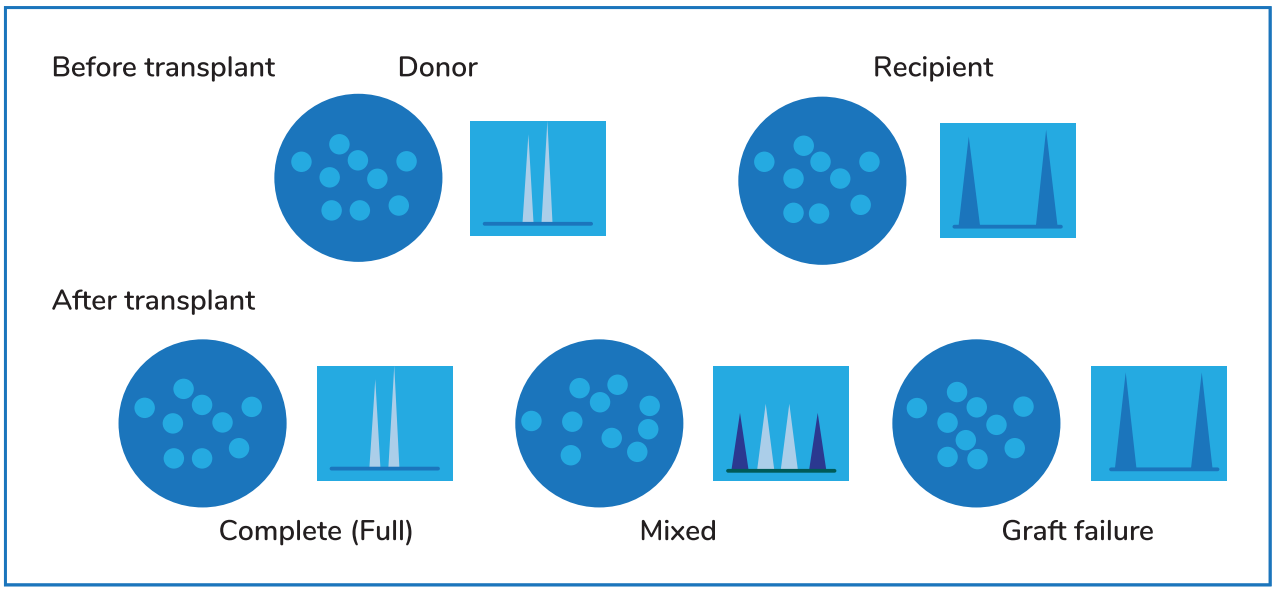
Allogeneic Hematopoietic Stem Cell Transplant (HSCT) is one of the best available treatment modalities for management of various malignant and non-malignant hematological disorders. The complete donor-derived hematopoiesis is essential for the sustained engraftment and for the prevention of the relapse of underlying disease. For this purpose, the molecular profile of polymorphic genetic markers, alone or in combination with other cell surface markers have proven very effective in detecting and quantifying the donor cells and their daughter cell types. This provides many useful information for therapeutic interventions and monitoring of the HSCT recipient patients.



Why Chimerism Testing?

Transplant monitoring by measuring the relative ratio of the recipient and the donor cell population in the recipient's post-transplant samples.

This provides several clinically actionable information for level of engraftment, rapid reduction/ optimization of immunosuppressive medications, administration of immunomodulatory cytokines, donor lymphocyte infusions (DLI) and other cellular therapies, early detection of disease relapse, Graft-versus-Host disease (GVHD)/Graft-versus-Tumour (GVT) effect etc.



Sample Requirement:

The Medgenome Chimerism analysis can be performed on both peripheral blood (PB) and/ or bone marrow aspirate (BMA). Since the bone marrow is the primary site of intervention here, it might appear intutive to consider BMA samples to be superior. However, the evidence base, at present, is not sufficient to make any technical recommendation of BMA over PB or vice versa for this test. We accept both the sample types.This analysis requires the pre-transplant* samples of both recipient and donor and post-transplant samples of the donor.

This analysis requires the pre-transplant* samples of both recipient and donor and post-transplant samples of the donor.

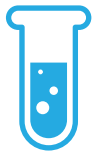
Pre-Transplant sample of (Patient/Recipient and Donor both):

2-4ml of peripheral blood in EDTA tubes

Samples should be stored and transported at ambient (15-25^{0}C) temperature

Ice packs should be used for transportation of samples in summers, especially in the areas where temperatures can reach > 35^0 C

Post-Transplant samples (Patient/Recipient only):



Patient:2-4ml peripheral blood or 1-2ml of Bone Marrow Aspirate in EDTA tubes

Samples should be stored and transported at ambient (15-25^{0}C) temperature

Ice packs should be used for transportation of samples in summers, especially in the areas where temperatures can reach > 35^0 C

*In case of Patient's pre-transplant samples are not available - four (4) Buccal swabs can be collected in Medgenome supplied kit

Methodology

The Chimerism testing employs on detection and comparison of DNA short tandem repeat (STR) markers in the recipient and donor before and after transplantation.

1. STRs are highly polymorphic DNA sequences in the human genome. The test samples along with a positive control DNA are subjected to multiplex STR typing in which different alleles present at 16 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, FGA)on chromosomes 2, 3, 4, 5, 8, 7, 11, 12, 13, 16, 18, 19, 21, X and Y are amplified in a single reaction and analyzed by capillary electrophoresis.
2. These loci exhibit alleles that may differ in length between individuals and are inherited as codominant Mendelian traits.
3. After confirming the presence of expected control markers, the most informative STR markers are used to differentiate and quantify donor and recipient components in the post-transplant sample.
4. The patient's post-transplant STR profile peaks are compared to the pre-transplant STR peaks of patient and donor to calculate the % donor or % recipient chimerism.

Different methods for Chimerism Testing

The Medgenome Chimerism Test evaluates the success of a HSCT and post-transplant monitoring by measuring the relative ratio of the recipient and the donor cell population in the recipient's post-transplant samples.

This provides several clinically actionable information for level of engraftment, rapid reduction/ optimization of immunosuppressive medications, administration of immunomodulatory cytokines, donor lymphocyte infusions (DLI) and other cellular therapies, early detection of disease relapse, Graft-versus-Host disease (GVHD)/Graft-versus-Tumour (GVT) effect etc

Salient features of the MedGenome Chimerism Test

- Medgenome uses 16 STR markers for analysis and ≥ 4 informative markers for comparison of results or reporting of a mean
- The limit of detection for our analysis is 1 %
- Automated software which normalizes the stutter peaks by a validated algorithm, It also gives:

  - Mean of individual locus estimates for % Chimerism (CHM) on a sample
  - Coefficient of variation (CE) for a sample
  - Locus error (LE) indicating the deviation of a single locus estimate from the sample mean
  - Sensitivity for detecting a minor component in a locus
  - DNA measurement error (ME) for gauging the platform's global performance for a locus

- External quality assessment (EQA/proficiency testing) done and the same accredited by College of American Pathologists. (Clark et al, 2014).

Turnaround Time

7 Working days from the order booking of the sample(s)

Report format showing STR profiles for Pre and Post Transplant samples

% Donor Chimerism

No	Maker Name	% D CHM	LE	ME	Ignored?
1	D8S1179	89.66%	8.44%	1.15%	NO
2	D21S11	96.70%	1.26%	7.41%	NO
3	D7S820	NI	NI	NI	YES (AUTO)
4	CSF1P0	NI	NI	NI	YES (SAI)
5	D3S1358	100.00%	2.11%	7.18%	NO
6	TH01	100.00%	2.11%	NAN	NO
7	D13S317	NI	NI	NI	YES (AUTO)
8	D16S539	100.00%	2.11%	9.68%	NO
9	D2S1338	99.53%	1.63%	NAN	NO
10	D19S433	100.00%	2.11%	23.78%	NO
11	VWA	100.00%	2.11%	NAN	NO
12	TPOX	NI	NI	NI	YES (AUTO)
13	D18S51	NI	NI	NI	YES (AUTO)
14	AMEL	NI	NI	NI	YES (AUTO)
15	D5S818	95.49%	2.50%	NAN	NO
16	FGA	NI	NI	NI	YES (SAI)

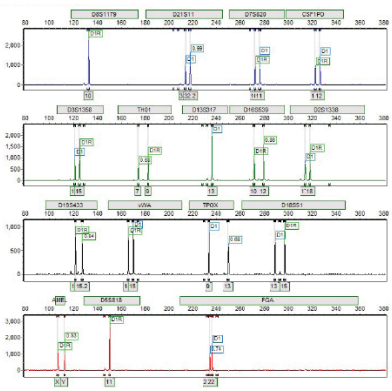
%D CHM
Average Chimerism:97.93%
Coefficient of Variation: 3.61%
St.Dev:3.53
MOE:2.72(95%)
Number of informative Loci:9

NI=Non Informative, SAI= Shared Allele Imbalance, LE= Locus Error, ME= Measurement Error

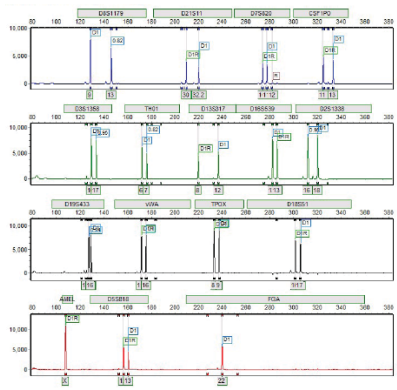
Report format showing STR profiles for Pre and Post transplant samples

POST-TRANSPLANT ENGRAFTMENT MONITORING

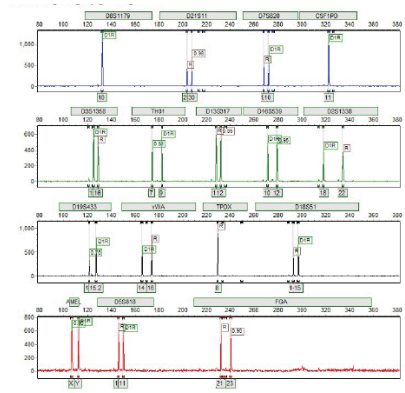
CASE-1



Patient



Donor



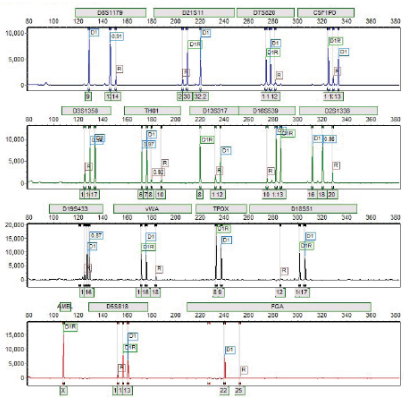
Post-transplant

Donor Chimerism : 99.86%

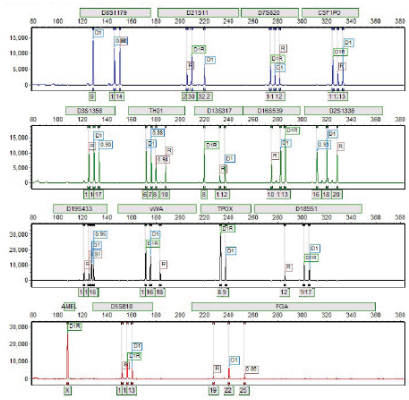
Recipient Chimerism : 0.14%

INTERPRETATION : 100% Donor chimerism is achieved in the post transplant sample of the patient

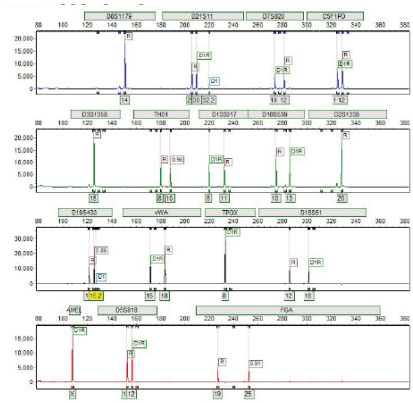
CASE-2



Patient



Donor



Post-transplant

Donor Chimerism : 89.74%

Recipient Chimerism : 10.26%

INTERPRETATION : 89.7% Mixed Donor chimerism is achieved in the post transplant sample of the patient

References

1. Ding-Ping Chen, MS; Kuo-Chien Tsao, BS; Po-Nan Wang, et al. Quantitative Analysis of Chimerism after Allogeneic Peripheral Blood Stem Cell Transplantation. Chang Gung Med J Vol. 25 No. 11, Nov, 2002.
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4. Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. Nature 1985; 316:76–9.
5. Jordan R. Clark, Stuart D. Scott, Andrea L. Jack et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. Br J Haematol. 2015 Jan;168 (1):26-37

Sample Report

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DNA TEST REPORT– MEDGENOME LABORATORIES

PRE-TRANSPLANT DETAILS:

PATIENT'S DETAILS		DONOR'S DETAILS	
Full Name / Ref No:		Full Name / Ref No:	
Order ID / Sample ID:		Order ID / Sample ID:	
Age / Gender:		Age / Gender:	
Date of Sample Receipt:		Date of Sample Receipt:	
Diagnosis:		Date of Report:	
Sample Type:		Sample Type:	
Referring Clinician:			

POST-TRANSPLANT DETAILS

Full Name / Ref No:		Transplant Date:	
Order ID / Sample ID:		Date of Sample Receipt:	
Test Requested:	POST BMT ENGRAFTMENT MONITORING BY STR TYPING (MGM …..)		

RESULTS

Donor Chimerism	89.74%
Recipient Chimerism	10.26%

INTERPRETATION

Mixed Chimerism is observed in the post-transplant sample of XXXXXXXXXXXXXX.

* Refer Annexure-I for detailed report

BACKGROUND

Allogeneic HSCT is considered to be the best treatment modality for various malignant and non-malignant hematological disorders. In human HSCT, complete donor-derived hematopoiesis is considered essential for sustained engraftment and for preventing relapse of the underlying disease. The genotypic profile of polymorphic genetic markers or those of their products in recipient and donor act as a specific tag to identify and quantify the presence of specific cell types in the post HSCT recipient. The transplant engraftment monitoring (chimerism) test evaluates the success of a hematopoietic stem cell transplant by measuring the relative ratio of the recipient and the donor cell population in the recipient's post-transplant specimen.

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