most homologous reference allele by intronic mismatches, most of which are single nucleotide polymorphisms in intron 1, likely explained by the previous lack of sequencing of this region. We have developed a full-length typing protocol for DQA1 and DPA1 and increased the number of available sequences accessible on the IPD-IMGT/HLA Database. We can study the effects of ultra-high-resolution DQA1 and DPA1 allele matching on HSCT, and in conjunction with our HLA-DQB1 and -DPB1 SMRT® sequencing this provides an opportunity to consider the genotypes encoding the HLA DQ and HLA DP molecules and their role in HSCT and solid organ transplantation.

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ASSESSING T LYMPHOCYTE ACTIVATION BY NON-CONVENTIONAL METABOLOMICS

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The current methodologies to evaluate T-cell function are based on very laborious or/and expensive methods. Nevertheless, the assessment of the appropriate functioning of these cells is very important in the clinical context, namely as a diagnostic tool of T-cell alloreactivity in different hematological settings, solid organ transplantation and immunosuppressive therapy. In the present work, we evaluated the potential of Non-conventional Metabolomics (NM) based on FTIR spectroscopy, in the assessment of T lymphocyte function after mitogen activation by phytohemagglutinin (PHa). T-cells isolated by magnetic-activated cell sorting, from seven heathy volunteers, were incubated for 1 hr at 37 °C with or without PHa, and subsequently dehydrated, and evaluated in minutes by NM. On the score-plot of principal component analysis (PCA), the first PC enabled the separation between resting (rT) and activated (pT) T-cells, pointing to a specific molecular signature of the two T-cells populations. The higher dispersion of rT-cell scores in relation to pT-cells scores, points to a higher variability of cellular metabolic states of rT-cells in relation to pT-cells. These observations were corroborated by hierarchical cluster analysis (HCA), where a clear discrimination between rT and pT-cell were obtained. The two distinct subgroups observed on the HCA dendrogram for the rT-cells, also corroborates the higher metabolic heterogenicity for these cells as observed in the PCA. In conclusion, the present approach enabled to discriminate resting from activated T-cells, in a simple, rapid, economic and high-throughput mode, therefore presenting a high potential for a new diagnostic tool of the T cell alloreactivity. This work was partially supported by Instituto Politécnico de Lisboa under grant IDI&CA/IPL/2018/RenalProg/ISEL.

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TARGETED GENE SEQUENCING FOLLOWING ENRICHMENT USING CAPTURE PROBES: THE NEXT GENERATION OF GENETIC MATCHING IN TRANSPLANTATION

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The human leukocyte antigen (HLA) genes are the most polymorphic loci in the human genome and encode immunoregulatory proteins that are critically important determinants of solid organ and haematopoietic stem cell transplant (HSCT) outcomes. Over the last 10 years, next generation sequencing (NGS) technologies following PCR amplification of HLA genes have revolutionised HLA genotyping by combining improved precision and gene coverage with the additional advantages of increased throughput capacity and reduced cost. Additional genes, including KIR and MICA, have also been shown to impact outcomes of HSCT and are being included in donor selection. However, as more genes are being identified, NGS workflows are becoming increasingly complicated. Furthermore, HLA gene coverage is limited by the location of amplification primers, meaning that important regulatory regions in untranslated regions may not be included in typing. We report an alternative protocol for NGS HLA typing using capture probes instead of PCR. The advantages of using capture probes are 1) complete gene sequencing is possible, beyond the sites used for PCR amplification, 2) the workflow is simple and capture can be performed for numerous samples in a single tube, 3) any gene or genetic region implicated in HSCT outcome, in addition to HLA, can easily be incorporated into the assay, and 4) the assay can be modified for high throughput HLA typing appropriate for registries. DNA library construction and hybrid capture are undertaken using Illumina Nextera Flex for Enrichment kits generating libraries suitable for sequencing on Illumina NGS instruments and sequence data is analysed in the sequence analysis software program, AlloSeq Assign. The use of capture probes for gene sequence enrichment represents a breakthrough in transplantation genetics that will enable better typed registries, improved matching of HSCT donors and patients, and help elucidate the role of non-HLA sequences in transplantation.