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Patient-specific Fluorescence Aberration Profiles during Longitudinal Clinical Trials

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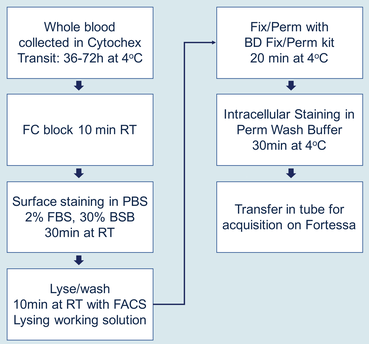
PURPOSE

Using a fully optimized multicolor whole blood, lyse/wash assay in a longitudinal clinical trial, abnormal, patient-specific profiles were observed in approximatively 10% of the patients. Extensive troubleshooting raised as many questions as answers.

METHODS



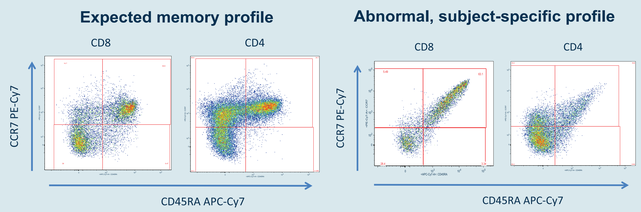
OBJECTIVE

To identify the source of the aberrant results by:

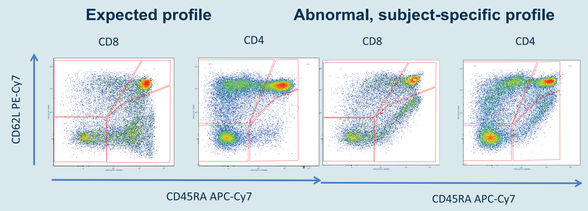
- Pre-washing the samples
- Changing the flurochromes

OBSERVATION

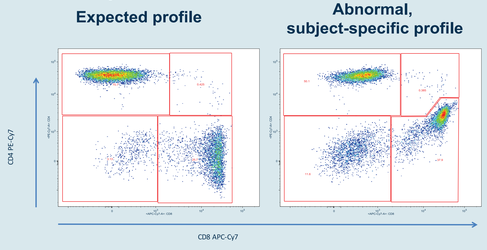
T cell Panel-1:

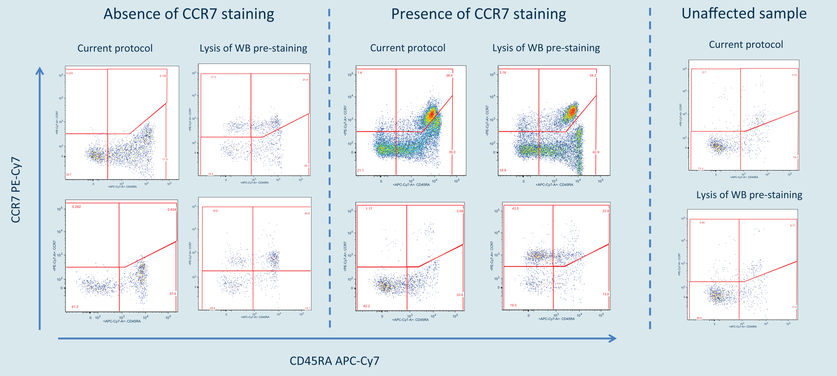


T cell Panel-2:



TBNK Assay:





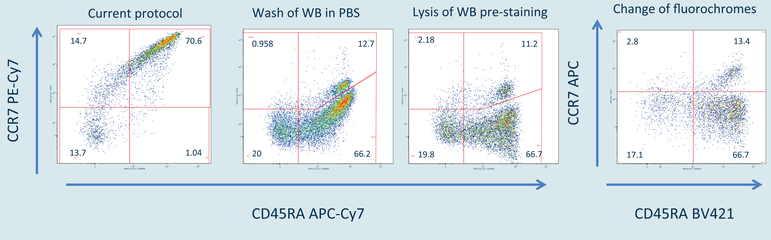
RESULTS

The presence of an aberrant CCR7-PE-Cy7 and CD45RA-APC-Cy7 double positive population was observed on approximatively 10% of the total subjects (n=40). This population was patient-specific and was observed during each visit. During troubleshooting, compensation values, pre-washing the whole blood, changing the fluorochromes, and comparing whole blood to PBMC were evaluated.

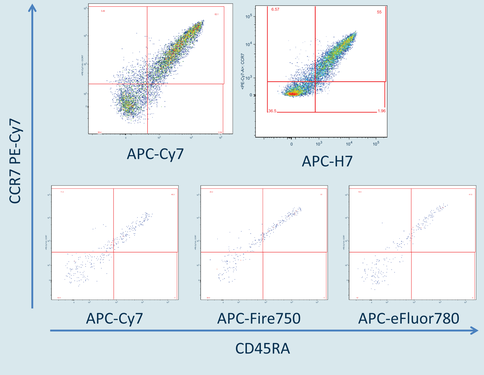
The aberrant population was not observed when:

- PBMC were stained instead of whole blood.
- The combination of CCR7-APC and CD45RA-BV421 were used.
- Only one of the tandem antibody was used
- The blood was pre-washed prior to staining with CCR7-PE-Cy7 and CD45RA-APC-Cy7.

Protocol Optimization:



APC-Cy7 alternatives testing:



CONCLUSIONS

Even with a fully optimized panel, the fluorochrome combinations may not be appropriate for every specimen. This adds to the challenges of using strictly set, validated panel in longitudinal clinical trials and highlights the difficulty of making the difference between a subject/treatment-specific immunophenotypic profile and an artefact caused by the choice of fluorochromes. Rigorous processes for post-examination data review are critical.

The lysis step is most likely not critical but allows for a complete wash of the plasma, or if panel composition allows, not to use APC-Cy7 (or similar variants) with PE-Cy7.

Future directions will include testing of whether the plasma is to blame for the issue (plasma spiking).

As we use more and more complex antibody panel, unexpected interaction between the different reagent and molecules present in the matrix may arise and will have to be accounted for. Pre-washing, or pre-lysing, whole blood samples may be an advisable stralegy.

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