Isolation of Antigen Specific CD8+ T cells Using The MACSQuant® Tyto™: A Closed, Sterile, Single-use Microchip-based Cell Sorter

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ABSTRACT

With Adoptive T Cell Therapies (ACTs) for cancer becoming more prevalent, the need for isolation and expansion of cells in a clinical setting is growing. The MACSQuant® Tyto™ (Miltenyi Biotec, Gmbh) is a bench top sorter that is fully closed, sterile and easy to use. The heart of the system is in the disposable, single-use, cartridge. This cartridge allows for completely aseptic sorting conditions with no chance of cross contamination between samples or patients. We demonstrated this capacity by using the MACSQuant® Tyto™ to sort cancer antigen specific cytotoxic T cells out of stimulated peripheral blood mononuclear cells (PBMCs). The desired T cells were labeled with PE conjugated tetramers. Sorted cells are transferred directly from the cartridge into expansion and later infused back into the patient in order to induce an immune response against that cancer antigen.

BACKGROUND

The MACSQuant® Tyto™ is Owl Biomedical's second generation Microchip-based cell sorting platform and has been conceived and produced in collaboration with Miltenyi Biotec. The data described in this poster demonstrates this system's unique ability to produce a clinical grade cell therapy product with minimal difficulty. We are currently in the process of obtaining FDA approval to transition this protocol from Owl Biomedical's first generation cell sorter (The Nanosorter®) to the MACSQuant® Tyto™ in a human clinical trial with metastatic melanoma patients at MD Anderson Cancer Center.

A

B

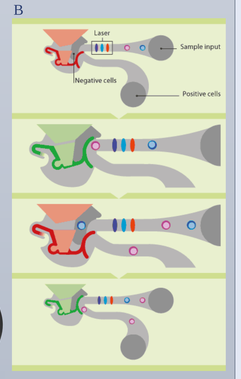

Figure 1. A. The MACSQuant® Tyto™ cell sorting platform including the disposable cartridge and the sorting Microchip B. Schematic of valve function within the microchip

MATERIALS & METHODS

A cancer patient's leukapheresis sample is depleted of CD25+ T cells and then stimulated with peptide pulsed antigen presenting cells (APCs). These samples are then labeled with the appropriate fluorescent-conjugated peptide-MHC tetramer to identify the desired antigen specific T cell population and sorted on the MACSQuant®Tyto™.

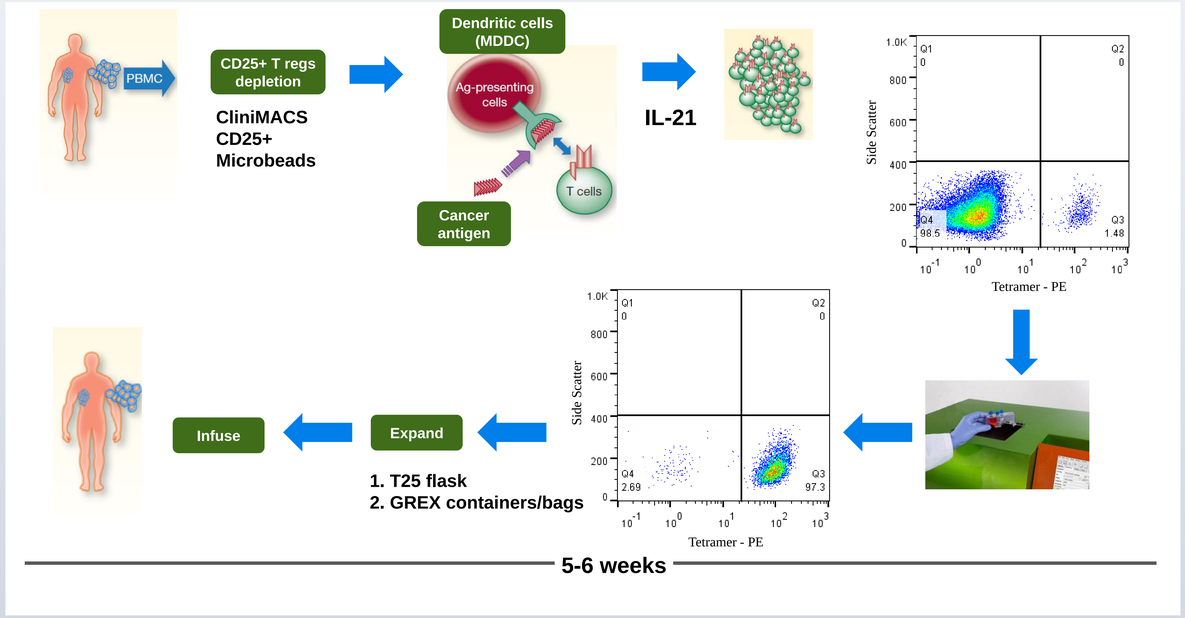

Figure 2. T cell immunotherapy protocol using the MACSQuant® Tyto™. The data shown in the figure was analyzed with the MACSQuant® Analyzer 10 (Miltenyi Biotec) and the dot plots were generated using FlowJo software. Sorting data is from the MACSQuant®Tyto™.

RESULTS - SORTING

Using the MACSQuant® Tyto™ we demonstrated reliable purification of cancer antigen specific cytotoxic T cells out of peripheral blood mononuclear cells with 6 separate samples. In samples 1-5 we sorted Mart-1 specific cells out of patient PBMCs (samples 1, 2 and 3) and a T cell line (samples 4 and 5). In sample 6 we sorted cells specific for a propriety cancer antigen from Immatics US, Inc. out of a T cell line. Although target populations in these 6 samples ranged from 0.35%-1.48%, results in all cases showed sort purities >88% (average 92.7±3.3%) and sort yields* >79% (average 85.2±5.5%). Sample concentrations ranged from $8.00x10^5$ to $1.89x10^6$ cells/mL.

*Sort yield = ((input%tetramer+ - unsorted%tetramer+) /(input%tetramer+))

Sample #	Input % Tetramer+ Cells	Sort Purity (%)	Sort Yield* (%)
1	1.48	97.0	85.6
2	1.44	96.2	86.8
3	1.29	90.2	85.3
4	0.60	91.1	80.0
5	0.48	88.9	79.2
6	0.35	92.6	94.3

Figure 3. Purity and yield results of sorting antigen specific T cells on the MACSQuant® Tyto™.

RESULTS – EXPANSION

After sorting, the cells are transferred directly from the cartridge into expansion. In a separate experiment, Ivy Lai et al. (MD Anderson Cancer Center) used the MACSQuant® Tyto™ to sort Mart-1(M27) antigen specific T cells out of 4 patient samples and then expanded the sorted cells for 12 to 15 days in antibiotic free medium. The sorted cells expanded to high numbers in this short time span while maintaining their antigen specificity.

Expansion #	% Tetramer+ Pre Expansion	%Tetramer+ CD8+ Post Expansion	Fold Expansion
1	86.8	46.6	1,636
2	88.4	84.2	5,988
3	80.5	80.0	5,117
4	59.7	72.9	18,546

Figure 4. Expansion Results of MACSQuant® Tyto™ Sorted Antigen Specific T Cells.

CONCLUSION

The MACSQuant® Tyto™ and its disposable sorting cartridge makes the process of generating antigen specific T cells for ACT reliable, easy and safe in a clinical setting. We have demonstrated consistent sort results with high purities and yields across a range of target frequencies with two separate cancer antigens. Furthermore, we demonstrated that the cells can be transferred directly from the cartridge into culture and expanded successfully in antibiotic free medium. In addition to the previously mentioned metastatic melanoma trial, MD Anderson Cancer Center has plans to expand the use of this protocol to treat Gastrointestinal cancers such as pancreatic and colon cancers. Finally, the production of GMP grade cartridges, running buffer, and CD8 antibody (all expected in 2017) will further facilitate GMP grade cell product manufacturing.

ACKNOWLEDGMENTS

Many thanks to the Yee Lab at MD Anderson Cancer Center and Immatics US, Inc. for providing the materials for these experiments.

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