Cell Mimics as Robust Positive Controls for Immunodeficiency Analyses

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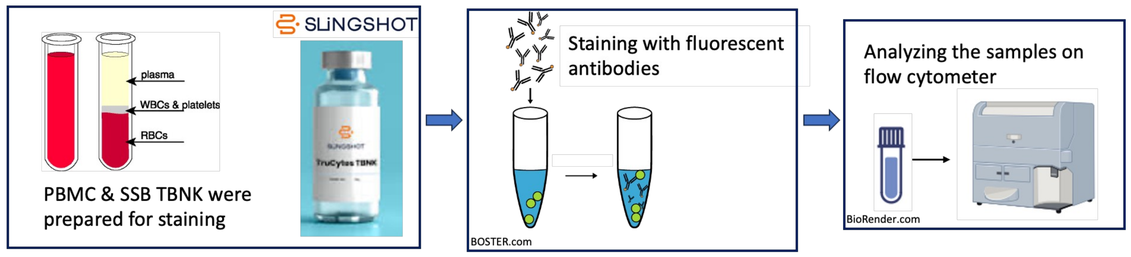
Abstract

The TBNK assay is valuable in various clinical and research settings to aid in the diagnosis and monitoring of immunodeficiencies, autoimmune diseases, and lymphoproliferative disorders. Stabilized blood samples, frozen cells, or lyophilized reagents are typically used as positive controls in TBNK assays. However, they are limited in terms of shelf life and storage conditions. Donor-to-donor heterogeneity and consistency of supply are also major challenges. To address the need for more flexible and consistent controls, Slingshot Biosciences has created cell mimics that contain a biologically relevant, heterogeneous mix of immune cell types for TBNK analysis that are a complete blood control without the drawbacks of donor-derived biologics. Here we show that our cell mimics match the optical scatter profile of PBMCs and whole blood. These cell mimics were also comparable to PMBCs and whole blood in terms of biomarker expression. Lot-to-lot consistency was superior compared to 3 PBMC donor lots. We also show that these cell mimics are stable over time and reproducible across labs, demonstrating inter-laboratory and inter-operator consistency. We have also engineered controls of different ratios of CD4/CD8 T cells (at levels of normal, HIV-positive, well-responded to ART samples, as well as those indicative of poor prognosis) and NK/ NKT cells (at levels of normal, with chemotherapy good prognosis, poor prognosis and with low survival rate). These controls can be in precise ratios to guide the clinical industry to diagnose diseases while avoiding biosafety risks, with batch to batch consistency and rapid turnaround time.

Methodology



Results

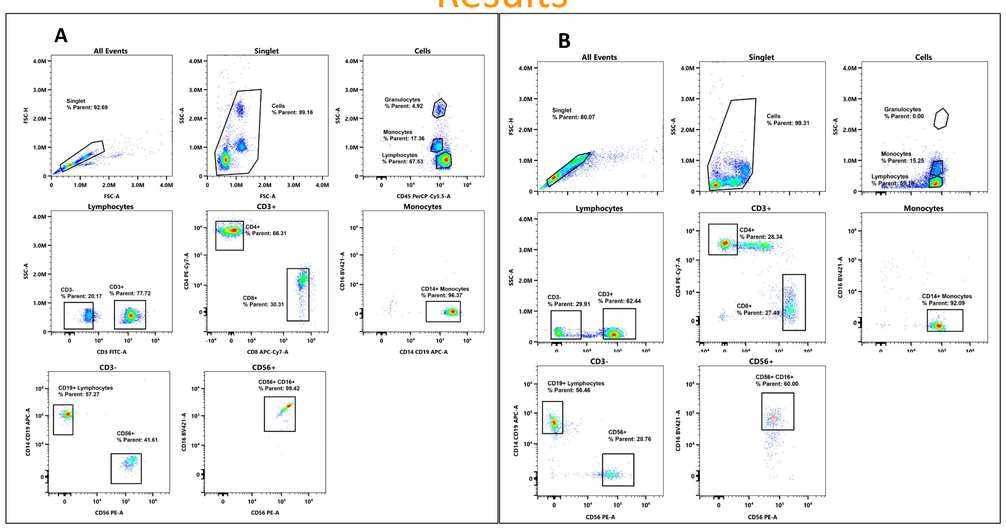


Fig 1. Slingshot TBNK Cell Mimics are comparable to PBMCs: Cryo-preserved PBMCs were thawed and stained with TBNK panel antibodies against Slingshot TBNK cell mimics. Samples were acquired on the Cytometer, gated and evaluated against each other. TBNK cell mimics **(A)** show overall scatter compatibility with PBMCs **(B)** as well as biomarker signals and positive % populations.

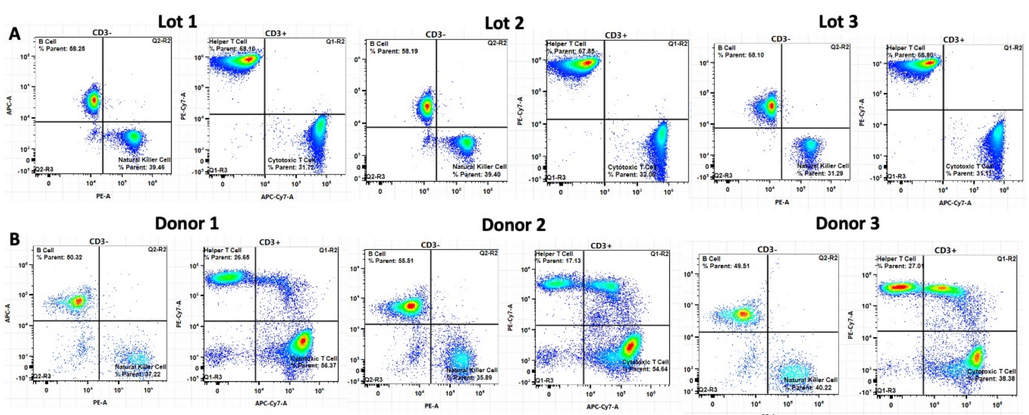


Fig 2. TBNK cell mimics' Lot-to-lot consistency against PBMCs' donor-to-donor's: Cryo-preserved PBMCs were thawed and stained with TBNK panel antibodies against Slingshot TBNK cell mimics. Samples were acquired on the Cytometer, gated and evaluated against each other. Example plots from CD3- and CD3+ parent populations are demonstrated here. TBNK cell mimics' lots **(A)** show to be more efficient in terms of gating, and with more consistent %populations in comparison with PBMC donors **(B).**

Results

	Populations' %CV			
	Of Singlet gate		Of each population's parent	
	%CV PBMC	%CV TBNK	%CV PBMC	%CV TBNK
Lymphocyte	9%	1%	9%	1%
CD3+	19%	1%	15%	0%
Helper T Cell	39%	5%	24%	4%
Cytotoxic T Cell	31%	7%	33%	8%
B Cell	30%	18%	32%	16%
Natural Killer Cell	25%	14%	23%	16%
Monocyte	34%	13%	34%	13%
Granulocyte	26%	21%	26%	21%

Fig 3. TBNK cell mimics' Lot-to-lot consistency vs PBMCs' donor-to-donor's: Cryo-preserved PBMCs were thawed and stained with TBNK panel antibodies against Slingshot TBNK cell mimics. Samples were acquired on the Cytometer, gated and evaluated against each other. Percentage CVs were calculated for the % populations between 3 lots or 3 donors, and major TBNK populations and markers are demonstrated here. TBNK cell mimics' lots show lower %CV of the % populations in comparison with PBMC donors. The PBMCs and TBNK were tested on 3 different donors and lots respectively.

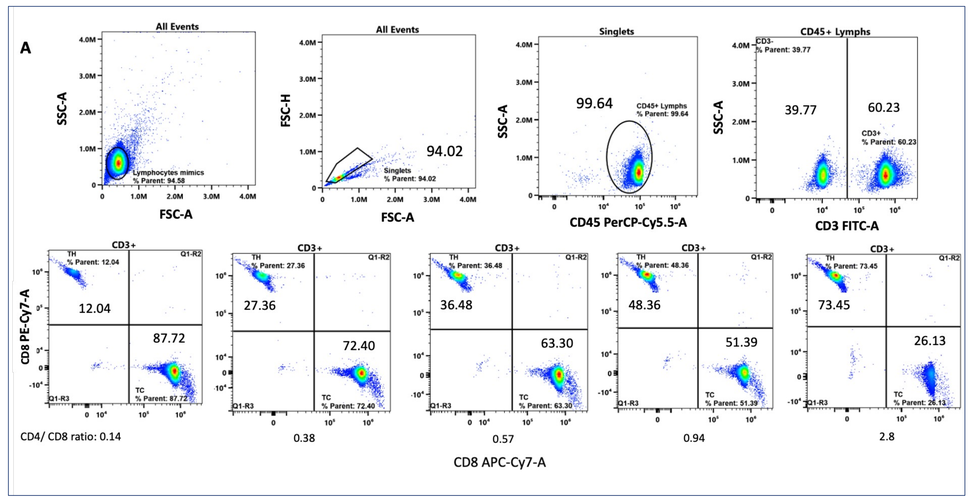
A

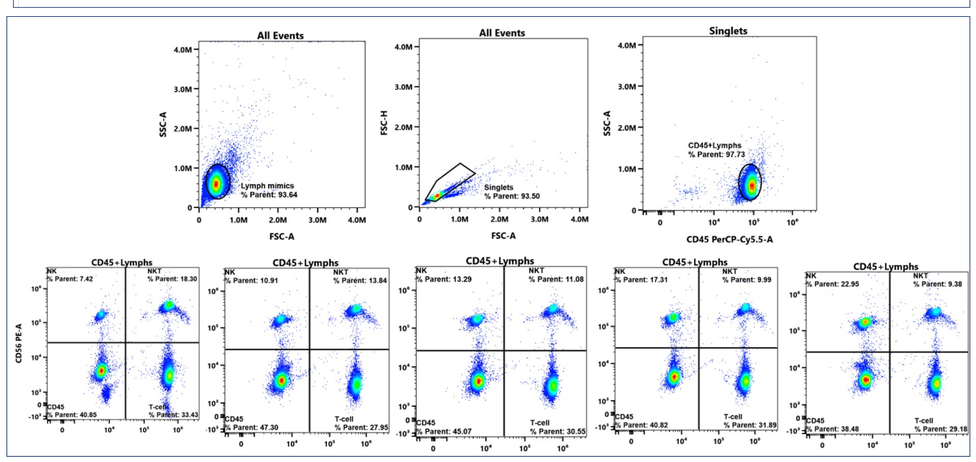
Clinically relevant conditions	CD4 particles	CD8 particles	CD4/ CD8 ratio
Uncontrolled HIV	~20k	~100k	0.2
Antiretroviral therapy HIV	~50k	~100k	0.5
Good prognosis	~70k	~100k	0.7
Healthy lower limit	~70k	~100k	1.0
Healthy upper limit	~120k	~100k	>1

B

Clinically relevant conditions	NK particles	NKT particles	NK/ NKT ratio
Low survival rate	~30k	~100k	0.3
Poor prognosis	~75k	~100k	0.75
Good prognosis	~100k	~100k	1.0
Healthy lower limit	~100k	~50k	2.0
Healthy upper limit	~137.5k	~50k	2.75

Fig 4. TBNK cell mimics to be utilized as clinical application controls. The custom built kits with clinically relevant ratios of specific cell subsets of TBNK cell mimics will enable the user to gauge patients' samples for disease prognosis and treatment follow-up. **A)** Clinically relevant CD4+/ CD8+ T cell ratios will assist HIV patients diagnosis, prognosis or treatment follow-up, analyzed **B)** Clinically relevant NK/ NKT cells ratio as customized subsets of TBNK cell mimic could assist chemotherapy follow-up and patients' prognosis. The final population percentages and ratios are calculated of only from CD4+ and CD8+ **(A)**, and CD56+ **(B)** parents.





Conclusion

We have shown that Slingshot Biosciences TBNK cell mimics are compatible with PBMCs' scatter properties, as well as TBNK commonly recruited panel. We also have demonstrated our cell mimics show higher %populations'' lot-to-lot consistency by showing lower %CV, in comparison to PBMCs. Furthermore, we have shown that custom-combinations of TBNK population subsets can be developed to represent clinically relevant ratios of CD4/CD8 and NK/NKT. These substitute cell mimics are non-biohazardous, with a longer shelf life and reliable supply compared to current biological samples.