ESTABLISHING A LOCAL BANK AND COMPARING EFFICACIES OF THIRD PARTY VIRUS-SPECIFIC CYTOTOXIC T CELLS (VST) AND CYTOKINE INDUCED KILLER CELLS (VSCIK)

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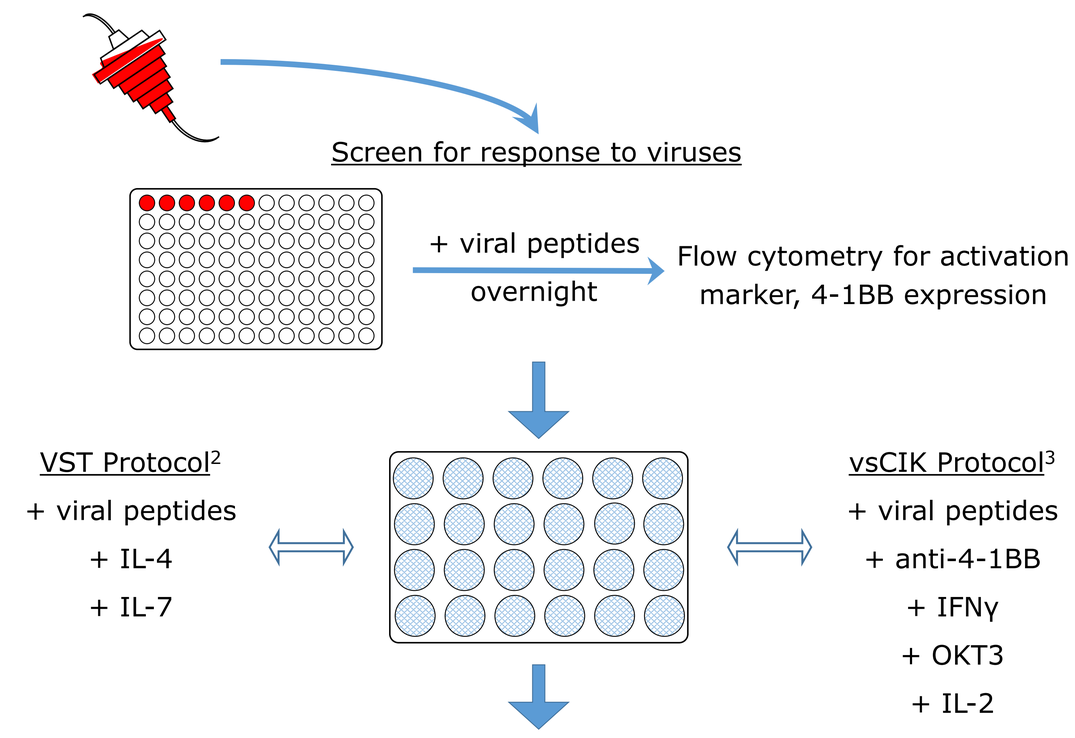
INTRODUCTION

Reactivation of dormant viruses is not uncommon after allogeneic haematopoietic stem cell transplants (allo-HSCT). Antiviral drug therapy is limited by refractory strains and toxicities, while matched donor derived VSTs have been successfully used to treat allo-HSCT patients with viral reactivation and there is promising data that minimally matched third party banked VSTs are also effective and safe. Here we explore the feasibility of a compact and effective bank of virus-specific cytotoxic cells for allo-HSCT patients.

MATERIALS AND METHODS

We (1) reviewed our database of the HLA class I types of 100 consecutive allo-HSCT recipients, (2) screened the local population for response to viruses of interest and (3) studied the anti-viral activities of VST and vsCIK cultures to determine the efficacies of VSTs versus vsCIKs.

Cones, which are normally discarded, from plateletpheresis of healthy donors were collected with consent. The PBMC in the cones were then screened for virus reactivity and if positive, further cultured following the vsCIK and VST protocols.



1. Cytotoxicity Assay
   - Cr^{51}-release assay using autologous PHA blasts with/without peptide loading as target cells
2. Degranulation Assay
   - flow cytometry analysis for CD107a expression in response to viral peptides, to quantify antigen-specific degranulation

CONCLUSIONS

We showed that our local population share common HLA alleles such that a compact third party bank from donors with these alleles can cover most patients. We also successfully generated AdV, BKV, CMV and HHV6-specific vsCIKs and VSTs. Interestingly, in the comparison between VSTs and vsCIKs for each virus, the results of the cytotoxicity assays and the degranulation assays do not always agree. Further screening of viral peptides (particularly for EBV) and mechanistic studies will be required to determine the more effective cell type for the proposed bank. We also do not dismiss the possibility that the more effective anti-viral cell type might be specific for each virus.

References

1. Population-specific Recombination Sites Within the Human MHC Region. Lam, TH, *et al*. Heredity 2013. 111(2): 131-138.
2. Activity of broad-spectrum T-cells as treatment for AdV, EBV, CMV, BKV and HHV6 infections after HSCT. Papadopoulou, A, *et al*. Sci. Translational Med. 2014. 6(242):1-1.
3. Characterization of the recognition and functional heterogeneity exhibited by cytokine-induced killer cell subsets against acute myeloid leukaemia target cell. Linn, YC, *et al*. Immunology 2012. 126(3):423-35.

Acknowledgements
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RESULTS

Table 1. Presence of common HLA class I alleles in allo-HSCT patients.
Amongst Singaporeans, 96% carry one or more of the 17 HLA class I alleles that have a frequency of > 5% in the local population.[1] Analysis of the HLA-A, B and C data of 100 consecutive allo-HSCT patients showed that 85% have three or more of these common alleles, while only 4% do not have any of them. It is, therefore, feasible to establish a compact panel of cytotoxic cells from donors with these common HLA alleles.

No. of alleles present	Chinese (n= 81)	Malay (n= 10)	Indian (n= 4)	Others (n= 5)	Total (n= 100)
0	0	3	1	0	4
1	1	0	2	1	4
2	5	1	1	0	7
3	20	4	0	2	26
4	21	0	0	1	22
5	20	2	0	0	22
6	14	0	0	1	15

Table 2. Number of donor samples that react positively to viral peptides during screening. A total of 14 donors were screened.

Virus	No. of positive donors
Adenovirus (AdV)	6
BK virus (BKV)	1
Cytomegalovirus (CMV)	7
Epstein barr virus (EBV)	0
Human herpesvirus 6 (HHV6)	6

Table 3. Comparison of functional activities of vsCIKs and VSTs.
For AdV, VST cells showed greater responses to viral peptides than the vsCIKs. However, there were incongruities between the cytotoxicity and degranulation comparisons for CMV and HHV6. So far, no donor cells have shown reactivity against EBV peptides. This is despite a high percentage of EBV carriers among Singaporeans and the successful use of the same peptides in published reports.

Virus	vsCIK > VST		VST > vsCIK		vsCIK ≈ VST	
	Cytotoxicity	Degranulation	Cytotoxicity	Degranulation	Cytotoxicity	Degranulation
AdV	0	0	3	4	1	0
BKV	0	0	0	1	1	0
CMV	4	2	0	3	3	2
HHV6	2	0	1	4	2	1

For the comparisons, differences of 10% or more were considered significant, while values below 5% were considered negative. For cytotoxicity, the values from wells with a ratio of 10:1 (effector:target) were compared.

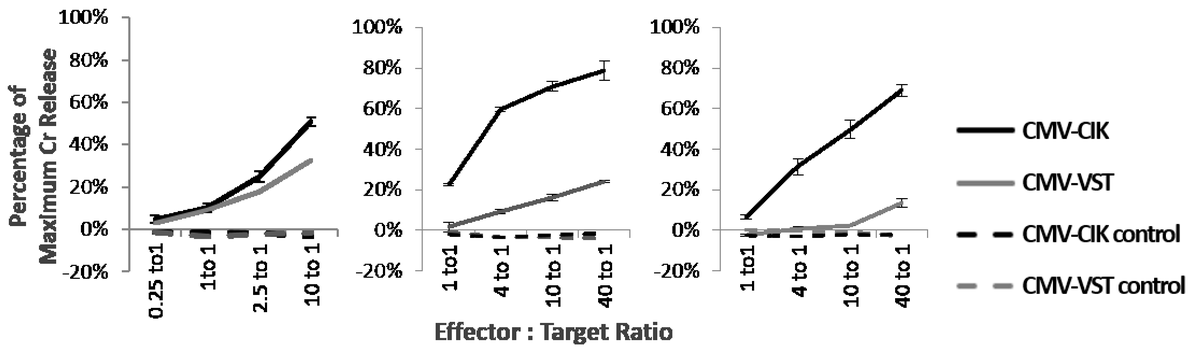

Fig 1. CMV-CIKs are more cytotoxic than CMV-VSTs.
VsCIKs consistently lysed more PHA targets loaded with viral peptides compared to VSTs. Results shown are from three separate experiments, each comparing CMV-CIKs with CMV-VSTs generated from the same donor.