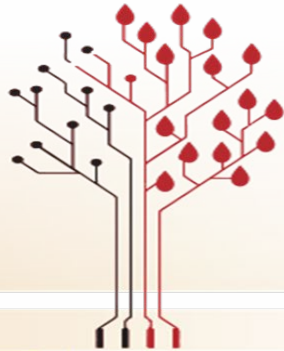




KIR GENOTYPING AT THE SOUTH AFRICAN NATIONAL BLOOD SERVICES (SANBS)

....first steps and looking ahead



**35th South African
NATIONAL BLOOD
Transfusion Congress**

5-8 August 2019 — Sun City

Adapt • Innovate • Advance

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BACKGROUND

- Natural killer (NK) cells have the ability to lyse target cells without prior sensitization (hence termed '**natural killer**' cells).
- NK cells are important in the innate immune system as they provide the **first line defence** against infectious agents.
- NK cells can **eliminate cells that fail to express self-MHC** molecules such as certain tumours.
- The functions of natural killer cells are controlled by families of antigen receptors. Prominent amongst these receptors are the **Killer cells Immunoglobulin-like Receptors (KIR)**.
- **KIR** receptors **regulates** the NK cell activity





INTRODUCTION

- Killer cells Immunoglobulin-like Receptors (KIR) is comprised of 14 distinct KIR genes and 2 pseudogenes located on chromosome 19q13.4.
- **Inhibitory KIRs**
 - ❖ 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3
- **Activating KIRs**
 - ❖ 2DS1, 2DS2, 2DS4, 2DS3, 2DS5, 3DS1
- **Pseudogenes**
 - ❖ 2DP1, 3DP1

KIR Nomenclature

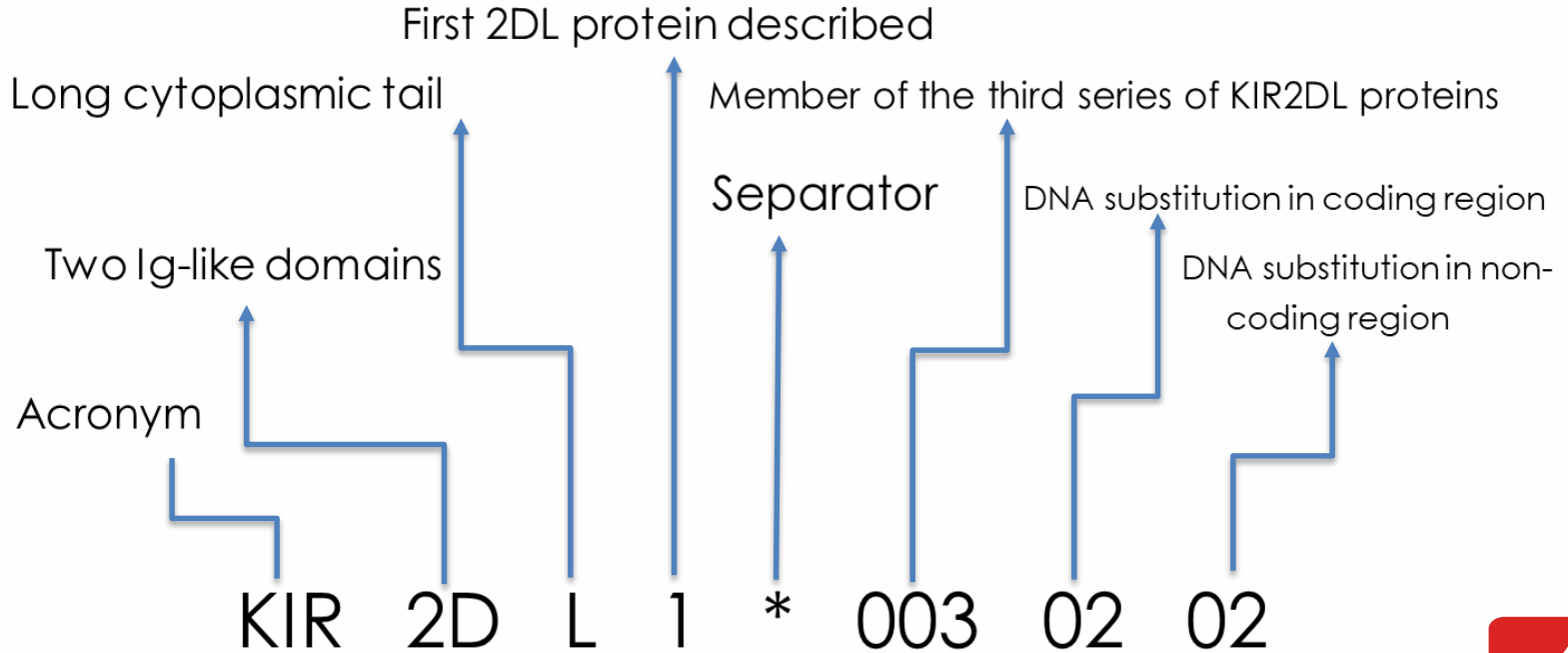
KIR and HLA

KIR Haplotype A and Haplotype B





KIR Nomenclature

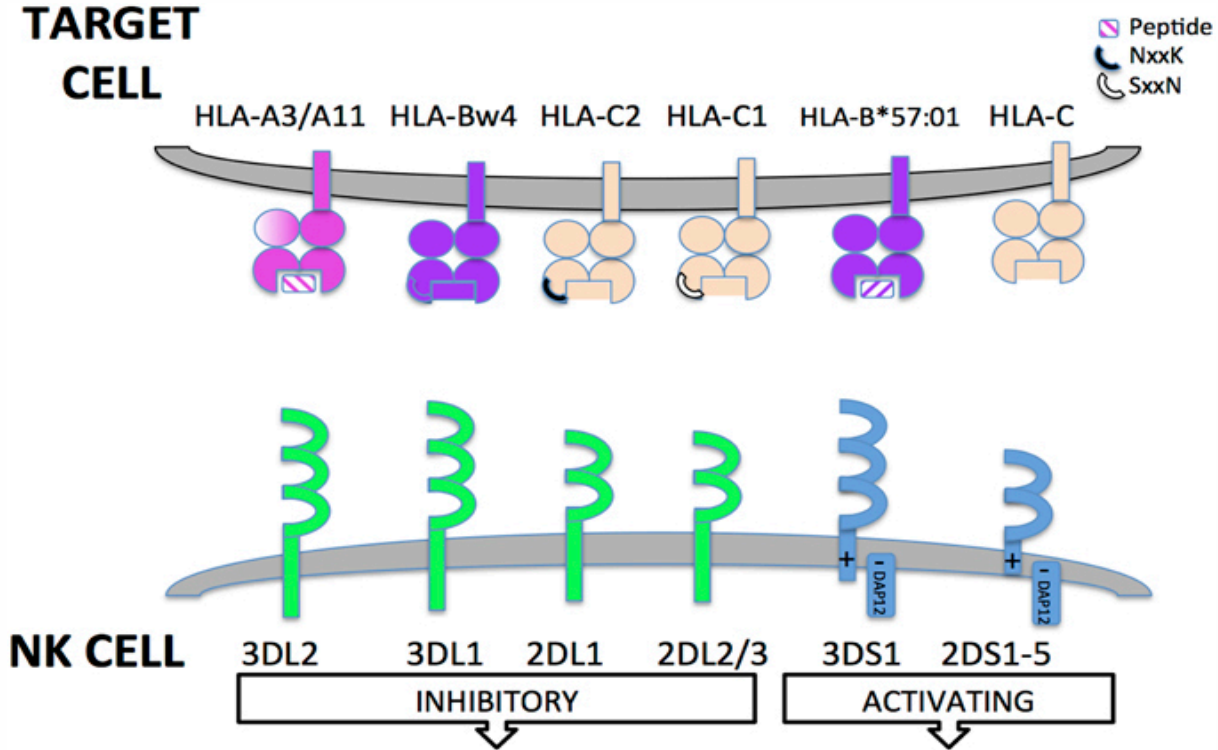


Middleton:2009



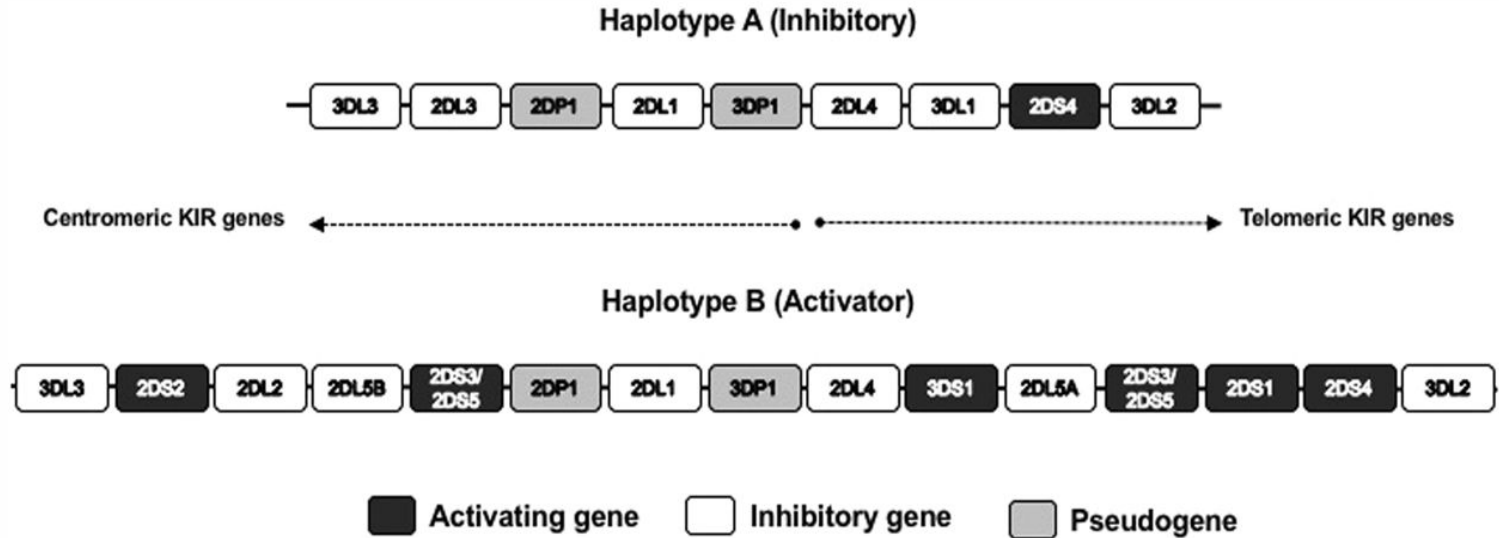


KIR and HLA





KIR - Haplotype A and Haplotype B





Methods

Innotrain KIR SSP Typing Assay



DNA EXTRACTION
(40 mins)

AMPLIFICATION
(90 mins)

GEL ELECTROPHORESIS
(20 mins)

ANALYSIS & INTERPRETATION (10mins)

	H	G	F	E	D	C	B	A
KIR	1	2	3	4	5	6	7	8
1	Negative Control 430 bp NG	2DL1 430 bp IC 105 bp	2DL2 430 bp IC 200 bp	2DL3 430 bp IC 130 bp	2DL4 norm 430 bp IC 140 bp	2DL4 deleted 430 bp IC 135 bp	2DL5 all 430 bp IC 130 bp	2DL5 (group 1) 430 bp IC 190 bp
4								
7								
10								
2	2DL5 (group 2) 430 bp IC 190 bp	2DL5 expressed 800 bp IC 315 bp	2DL5 null 430 bp IC 315 bp	2DS1 430 bp IC 105 bp	2DS2 430 bp IC 195 bp	2DS3 430 bp IC 85 bp	2DS4 norm 430 bp IC 175 bp	2DS4 (del-22bp) 430 bp IC 175 bp
5								
9								
11								
3	2DS5 430 bp IC 90 bp	3DL1 430 bp IC 200 bp	3DL2 800 bp IC 120 bp	3DL3 430 bp IC 140 bp	3DS1 430 bp IC 215 bp	2DP1 430 bp IC 130 bp	3DP1 variant (3DP1 variant) 3DP1 norm (1732 bp) 430 bp IC 280 bp	3DP1 variant 975 bp 430 bp IC
6								
9								
12								
	9	10*	11*	12	13	14	15	16
	17	18*	19	20	21	22	23*	24*



SANBS KIR Validation Results

	INHIBITORY								ACTIVATING					PSEUDOGENES		
	KIR2DL1	KIR2DL2	KIR2DL3	*KIR2DL4	KIR2DL5	KIR3DL1	*KIR3DL2	*KIR3DL3	KIR2DS1	KIR2DS2	KIR2DS3	KIR2DS4	KIR2DS5	KIR3DS1	*KIR3DP1	KIR2DP1
Sample 1																
sample 2																
Sample 3																
Sample 4																
Sample 5																
Sample 6																
Sample 7																
Sample 8																
Sample 9																
Sample 10																
Sample 11																
Sample 12																
Sample 13																
Sample 14																
Sample 15																
Sample 16																
Sample 17																
Sample 18																
Sample 19																
Sample 20																
Sample 21																
Sample 22																
Sample 23																
sample 24																
Sample 25																
Sample 26																
Sample 27																
Sample 28																
Sample 29																
Sample 30																

*Framework genes

KEY: Gene is present Gene is absent





KIRs in South Africa – pilot study

	INHIBITORY								ACTIVATING						PSEUDOGENES	
	KIR2DL1	KIR2DL2	KIR2DL3	KIR2DL4	KIR2DL5	KIR3DL1	KIR3DL2	KIR3DL3	KIR2DS1	KIR2DS2	KIR2DS3	KIR2DS4	KIR2DS5	KIR3DS1	KIR3DP1	KIR2DP1
000729	█	█		█		█	█	█	█	█	█	█	█	█	█	█
000606	█			█		█	█	█	█	█	█	█	█	█	█	█
000993		█	█		█		█		█		█	█	█	█	█	█
000517	█	█		█		█	█	█		█	█	█		█		
000592	█		█	█		█	█	█				█			█	█
000020	█	█		█	█		█	█	█	█	█		█	█	█	█
000371	█		█	█		█	█	█					█		█	█
000921	█		█	█		█	█	█				█		█	█	█
000614	█		█	█		█	█	█	█	█		█	█		█	█
000613	█	█	█	█		█	█	█		█	█	█			█	█
000916	█	█		█	█	█	█	█	█	█	█	█	█	█	█	█

Figure 1 - KIR genotypes in the South African population

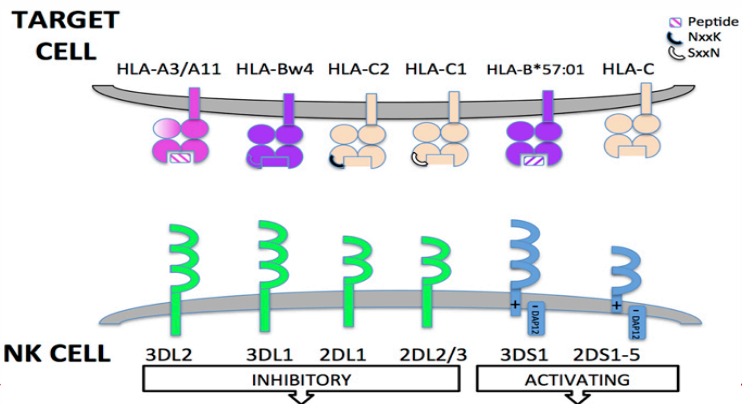
KIR framework genes





KIR and HLA – SANBS

INHIBITORY								ACTIVATING					PSEUDOGENES		HLA				
KIR2DL1	KIR2DL2	KIR2DL3	KIR2DL4	KIR2DL5	KIR3DL1	KIR3DL2	KIR3DL3	KIR2DS1	KIR2DS2	KIR2DS3	KIR2DS4	KIR2DS5	KIR3DS1	KIR3DP1	KIR2DP1				
█	█		█	█	█	█	█	█	█	█	█	█	█			A3/11	B35/57:01	Cw4Cw6	C2
█	█		█	█	█	█	█	█	█	█	█	█	█			A3/11	B35/57:01	Cw4Cw6	C2
█			█	█	█	█	█		█	█	█	█	█			A2/11	B13:01/40	C-C-	C
█		█	█	█	█	█	█		█	█	█	█	█			A3/11	B07/35	Cw4,C-	C2
█		█	█	█	█	█	█		█	█	█	█	█			A29/30	B13:02/42	Cw6, C-	C
█		█	█	█	█	█	█		█	█	█	█	█			A2/33	B39/44	Cw2Cw7	C2
█		█	█	█	█	█	█		█	█	█	█	█			A24/24	B40/44	Cw5, C-	C
█	█	█	█	█	█	█	█		█	█	█	█	█			A2/72	B15/15	Cw2Cw2	C2
█	█	█	█	█	█	█	█		█	█	█	█	█			A68/68	B15/58	Cw10Cw6	C
█	█	█	█	█	█	█	█		█	█	█	█	█			A1/2	B*51:01,*55:01	Cw1, C-	C1





DISCUSSION/CONCLUSION

Prognostic role of KIR genes and HLA-C after hematopoietic stem cell transplantation in a patient cohort with acute myeloid leukemia from a consanguineous community Gaafar et al

One of the first large studies identified that KIR2DL3, when present on a homozygous ligand background (HLA-C1/C1), was associated with spontaneous resolution of HCV infection (Khakoo et al., 2004)

KIR and HLA Haplotype Analysis in a Family Lacking the KIR 2DL1-2DP1 Genes Vojvodić S

*Killer cell immunoglobulin-like receptor 3DL1 variation modifies HLA-B*57 protection against HIV-1. Martin MP et al*

KIR Gene Content Diversity in a Zimbabwean Population: Does KIR2DL2 Have a Role in Protection Against Human Immunodeficiency Virus Infection? Mhandire K

KIR2DS4 promotes HIV-1 pathogenesis: new evidence from analyses of immunogenetic data and natural killer cell function Merino AM et al





CONCLUSION

- While there has been great advances in KIR genetics, knowledge of basic KIR biology (expression, specificity, function) lags well behind.
 - This is partly due to the difficulties in developing specific reagents for proteins with a high degree of similarity.
 - Experiences in interpretation and analysis of the repertoire of 15 KIR genes is also critical
- Looking ahead...

KIR – HLA → stem cell transplant

KIR – HLA - HIV → elite controllers vs acute vs chronic infection





ACKNOWLEDGEMENTS

- Angeline Moonsamy, Lavendri Govender, Morne Toms – KIR testing
- Dr Ute Jentsch – Lead Consultant for SLS
- Innotrain/Haemotec – support and assistance with interpretation and analysis

REFERENCES

- Innotrain website (www.innotrain.com), literature
- Vilches and Parham, 2002
- Moesta et al., 2008
- Gardiner, 2008
- Khakoo et al., 2004
- Middleton and Gonzelez, 2009

