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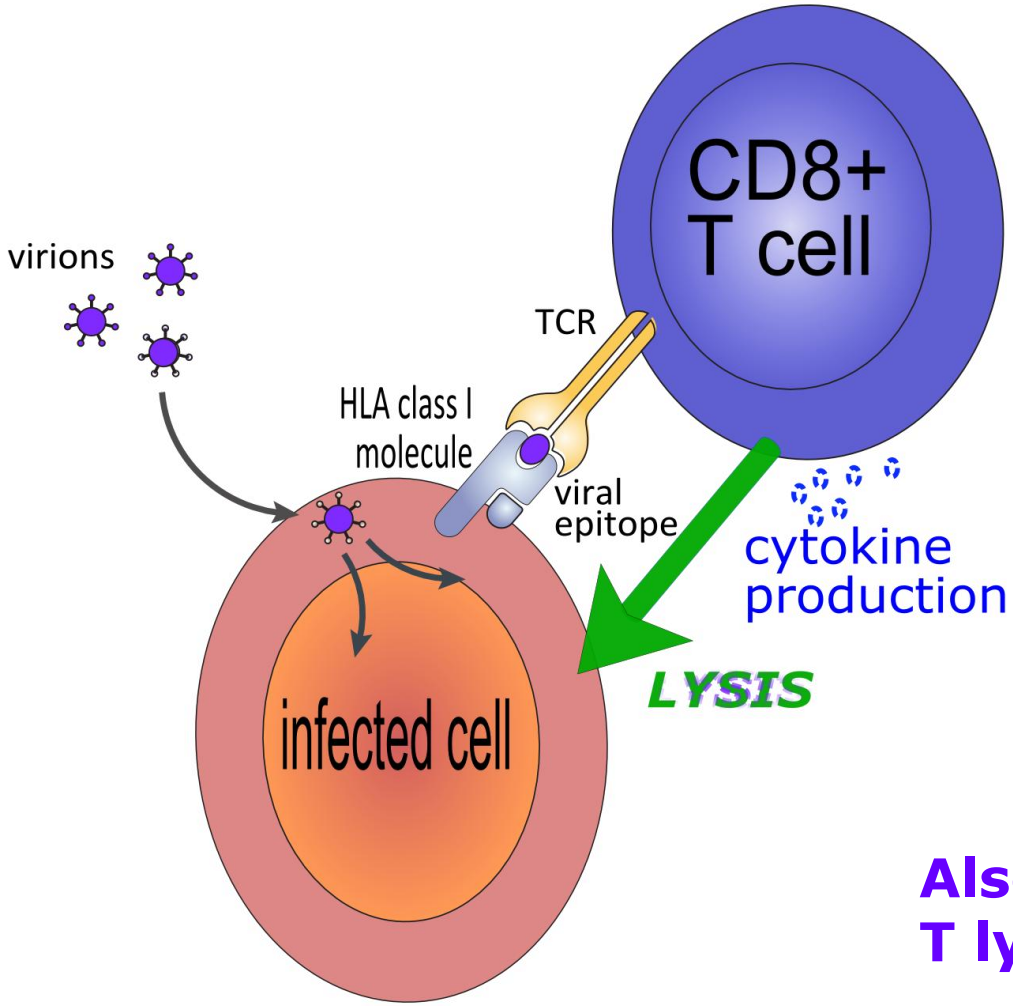
Efficiency of the $CD8^+$ T cell response to persistent viral infection

Becca Asquith

Background: CD8+ T cells



- Immune cells
- Help control virus infection
- Kill virus-infected cells

Also known as cytotoxic T lymphocytes (CTL)

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Research Questions

$CD8^+$ T cell Efficiency:
(in humans, *in vivo*)

Quantification.

Determinants.

Improvement.

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Determinants of $CD8^+$ T Cell Efficiency

What makes a good $CD8^+$ T cell response?

What makes a good HLA class I molecule?

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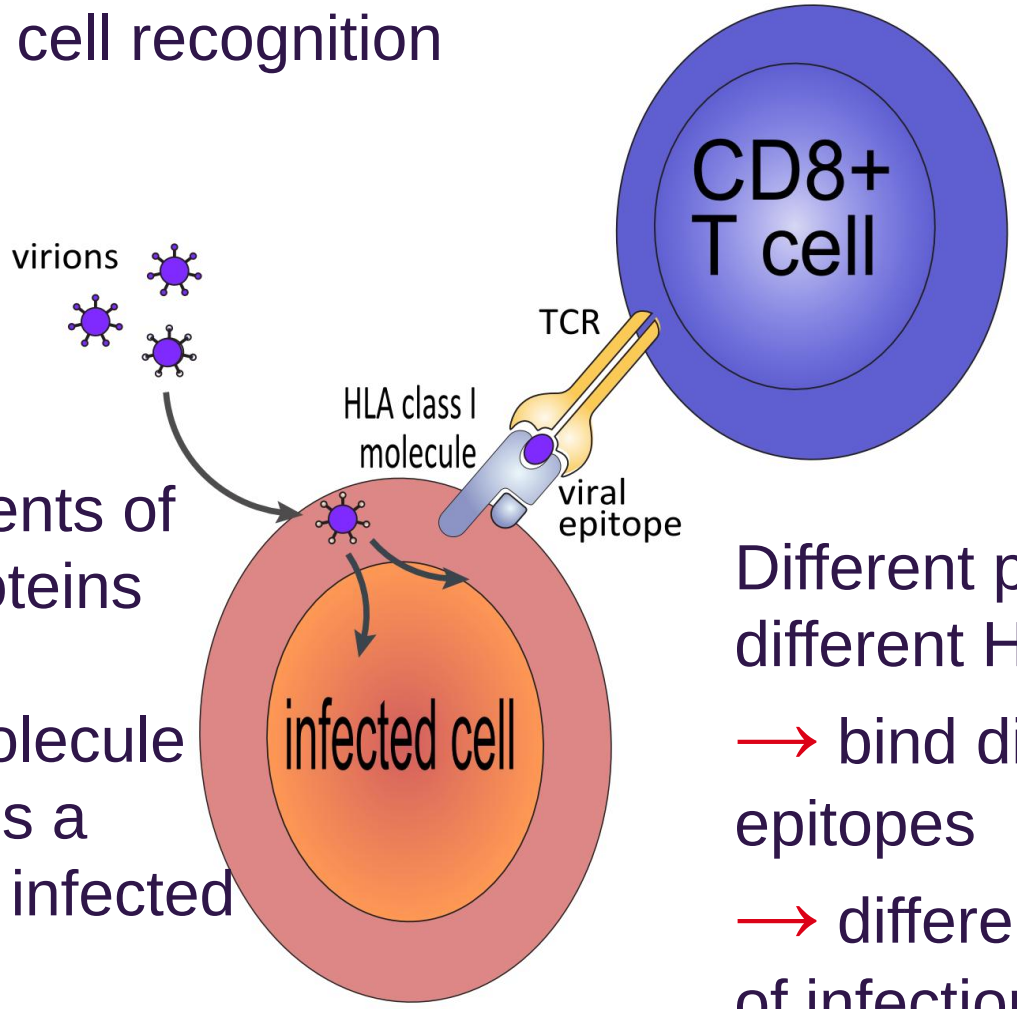
Background: HLA class I molecules

- Essential for T cell recognition



- Display fragments of intracellular proteins
- HLA class I molecule + viral epitope is a signature of an infected cell

Different people have different HLA alleles

→ bind different viral epitopes

→ different outcomes of infection

Determinants of $CD8^+$ T Cell Efficiency

What makes a good HLA class I molecule?

- Protein specificity
- KIRs

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What constitutes a protective HLA class I molecule?

Hypothesis:

Specificity is a determinant of HLA “protectiveness”

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Epitope prediction software (Metaserver)

Validated for known epitopes: HIV, HCV, EBV etc

HIV: 661 epitopes amongst 122,339 9-mers

SYFPEITHI: 148 epitopes amongst 782,442 9-mers

Sensitivity: 80%. Specificity: 97.4%

Significantly better than the best existing software ($P<0.00001$)

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Epitope prediction software

Tested for HTLV-1:

Synthesised 200 HTLV-1 peptides

Measured binding affinity (ELISA with a conformation dependent anti-HLA antibody)

A*0201 A*2402 B*0701 B*3501

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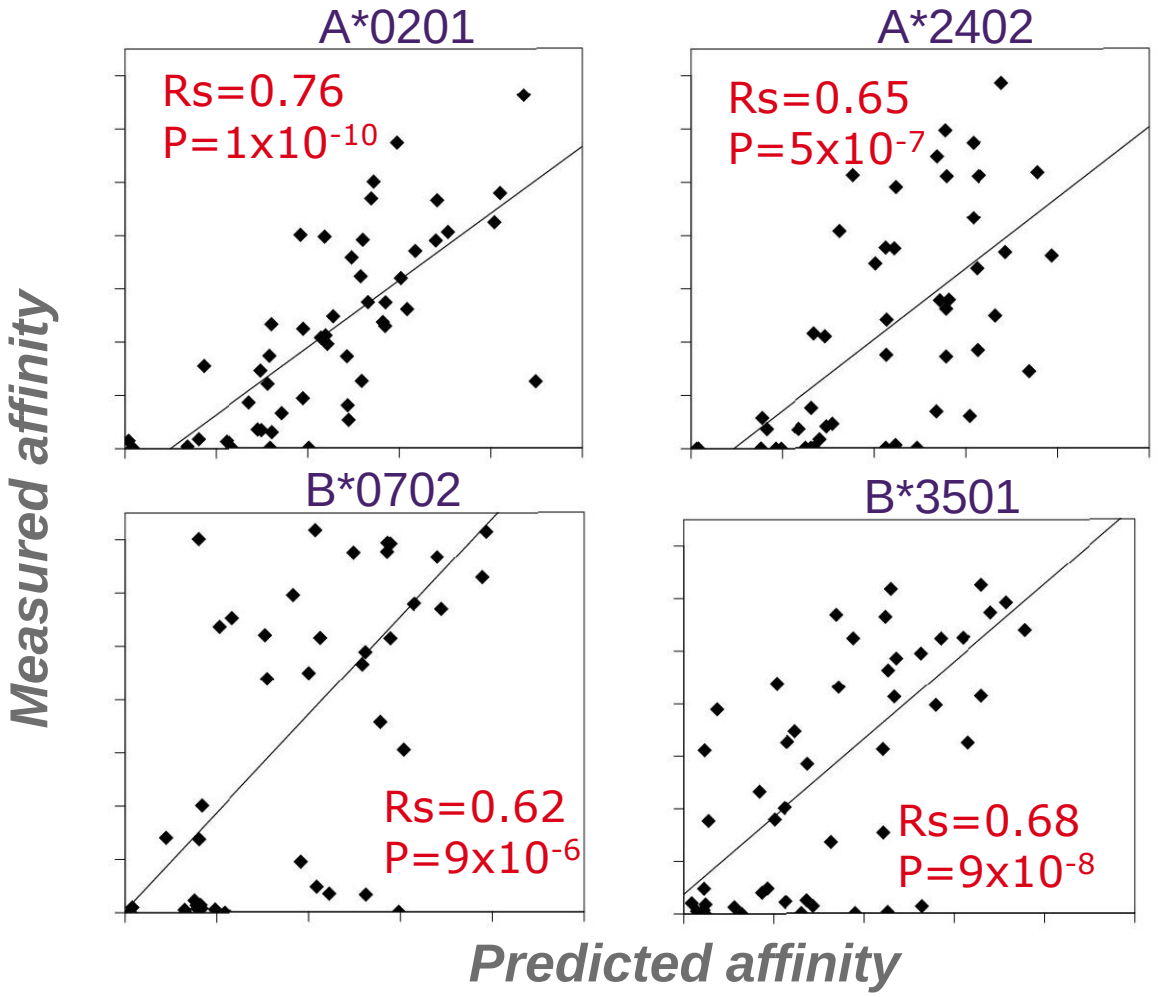
Epitope prediction software



$P=3x10^{-25}$

MacNamara et al PLoS Comp Biol 2009

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Epitope prediction software

Metaserver predicts HLA class I epitopes & binding affinity with good accuracy

MacNamara et al PLoS Comp Biol 2009

http://web.bioinformatics.ic.ac.uk/metaserver/
http://www1.imperial.ac.uk/medicine/people/b.asquith/

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Human T Lymphotropic Virus (HTLV-1)

Infects 10-20 million

95% Asymptomatic carrier (AC)

1-2% HAM/TSP

2-5% Adult T cell Leukemia

HLA-A*02, C*08 protective
B*54 detrimental

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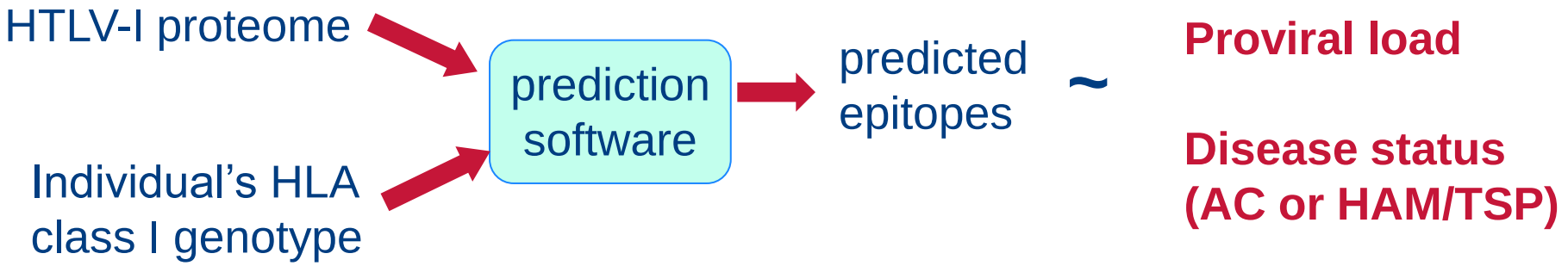
Method

Cohort of HTLV-I infected individuals from Japan

- 202 asymptomatic carriers (ACs)
- 230 HAM/TSP patients



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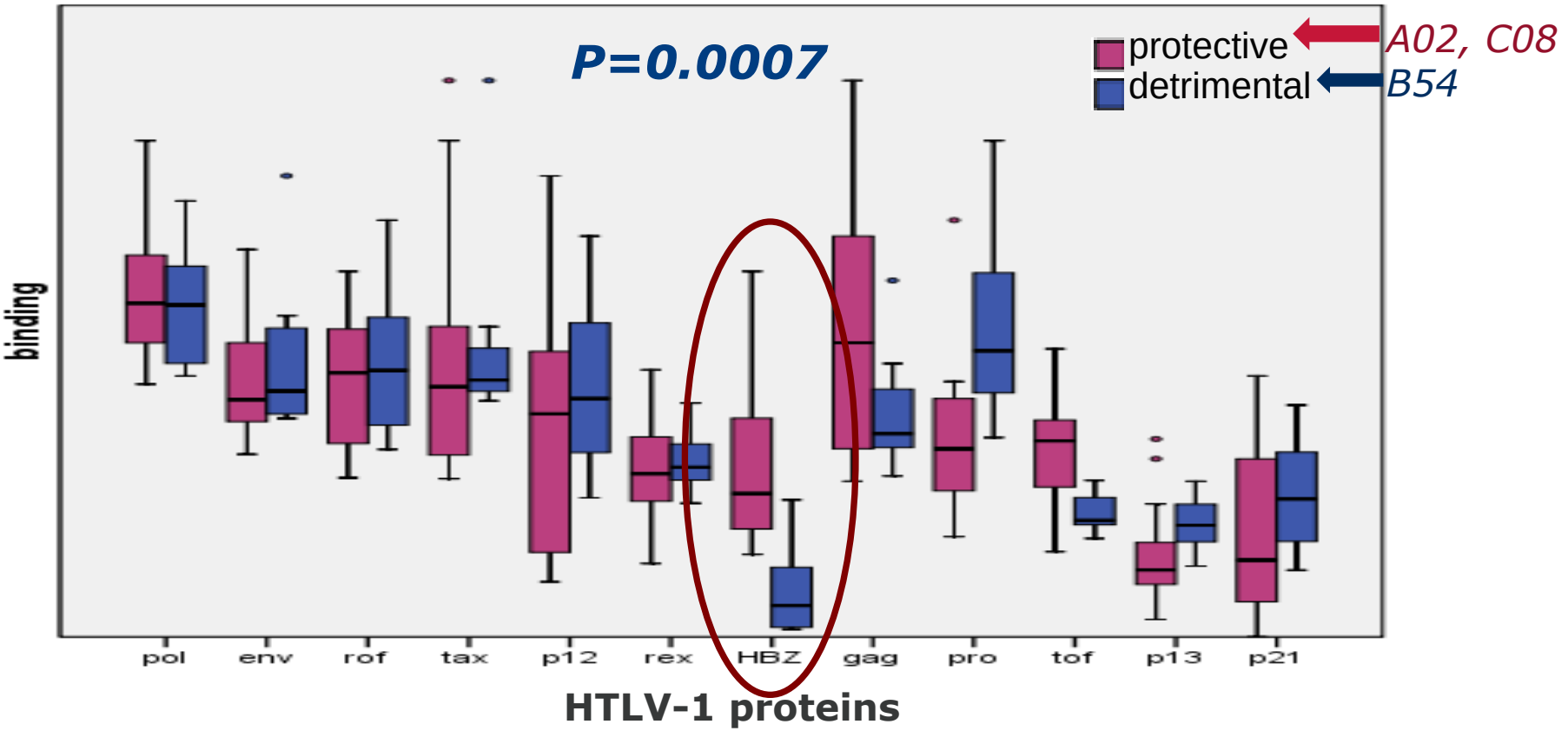
Do protective HLA molecules bind different proteins?



Protective HLA class I molecules bind HBZ strongly

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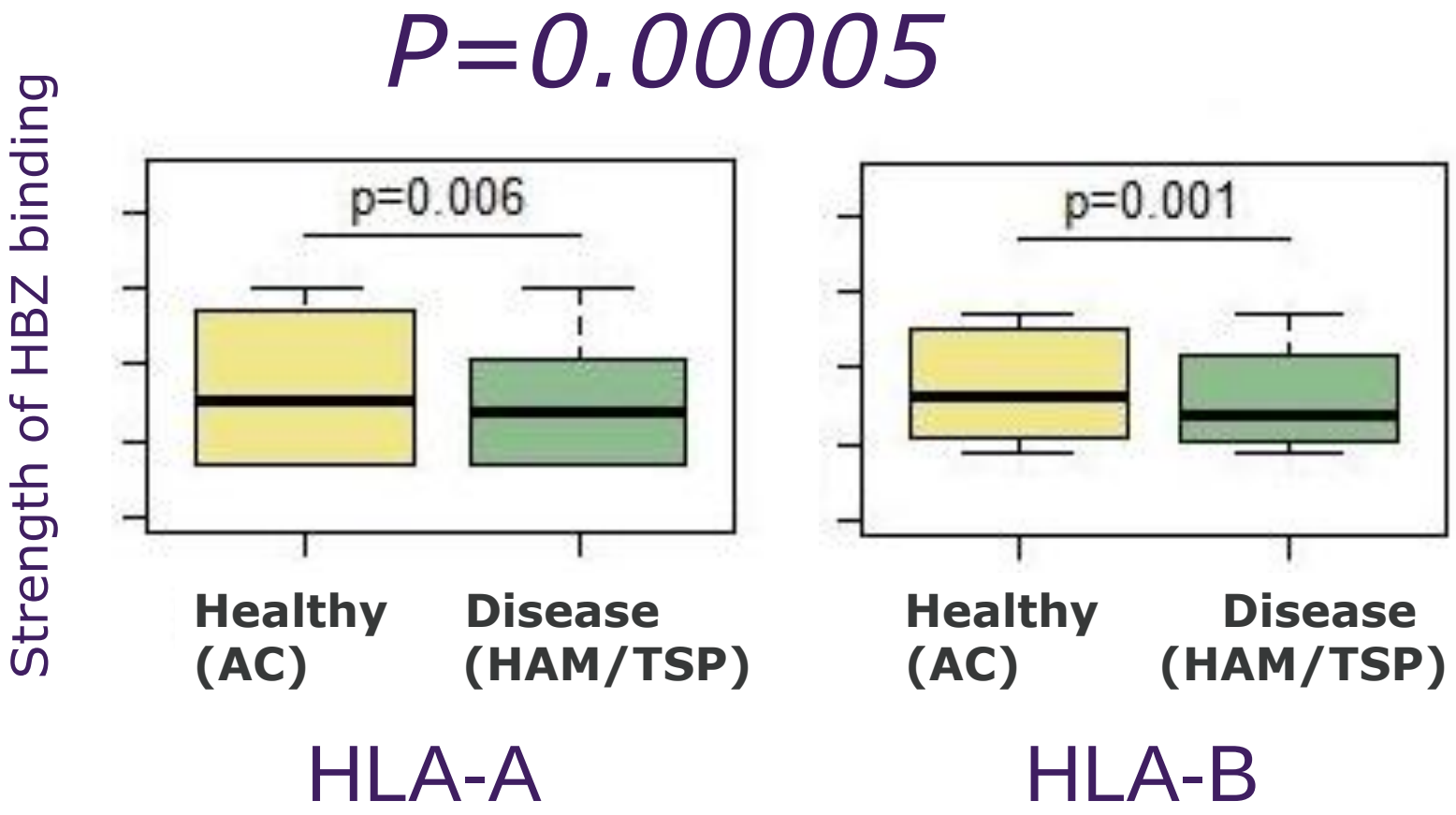
Do HAM/TSPs & ACs bind different proteins?



Asymptomatic carriers possess HLA molecules that bind HBZ strongly

Remove individuals with A2 or B54: result still holds

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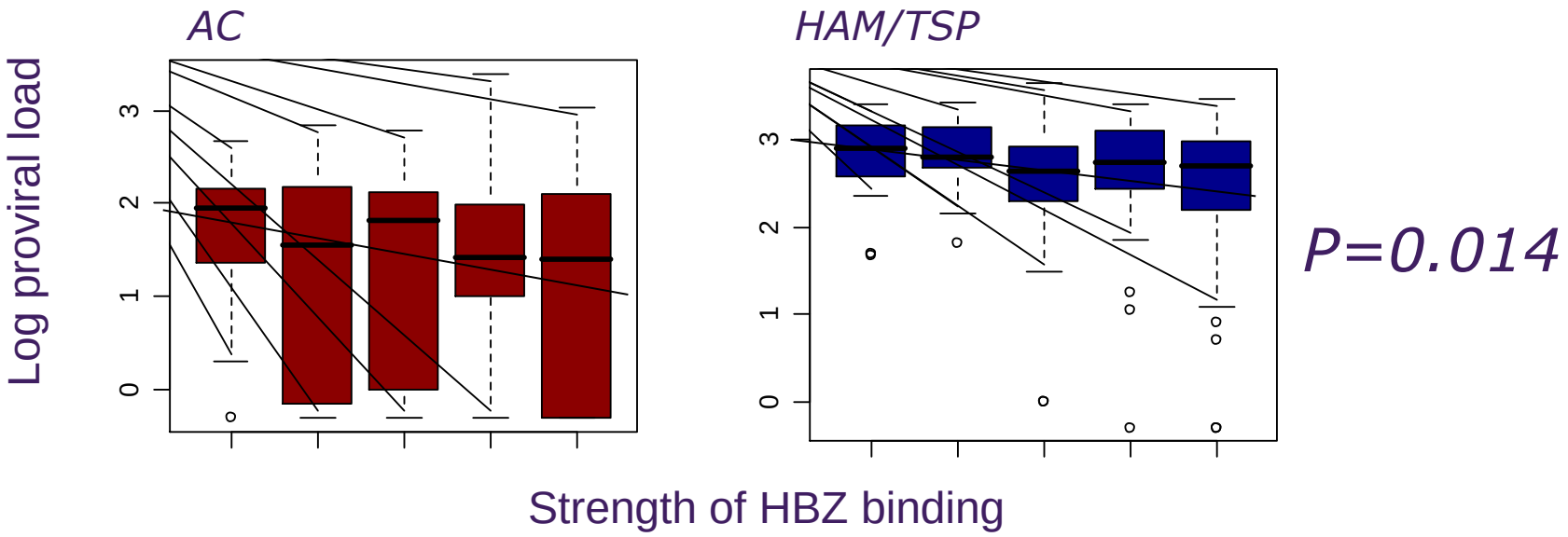
Does HLA specificity determine proviral load?



Strong binding of HBZ is associated with a low proviral load

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What makes a good HLA class I molecule?

HBZ
gag
p21
pol
p27
tax
rex
p30
p13
pro
p12
env

Bears no relationship to immunodomminance hierarchy

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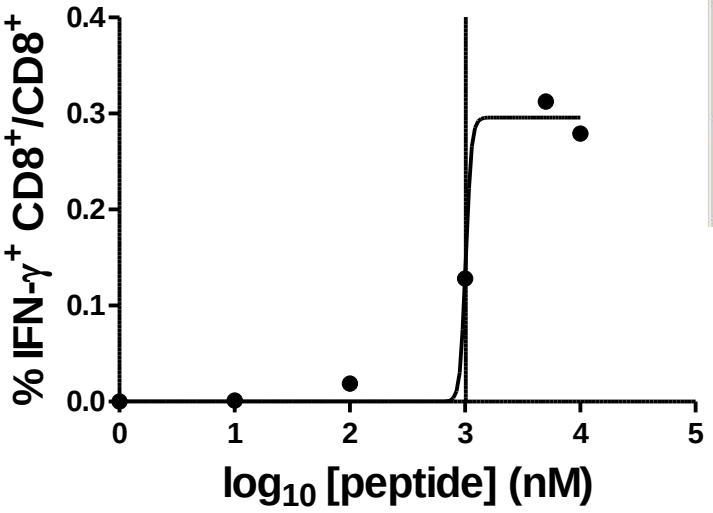
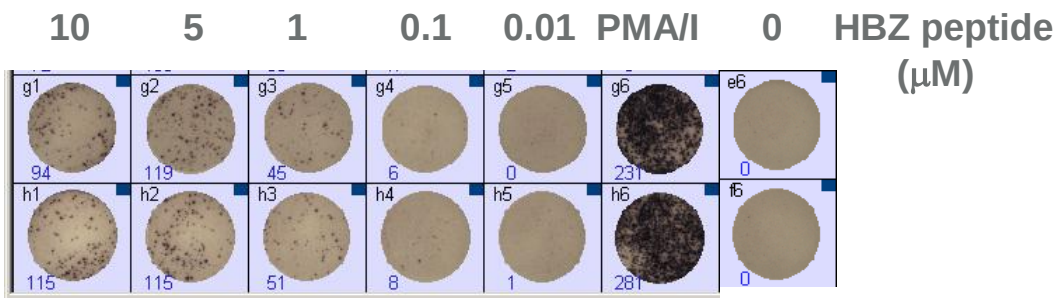
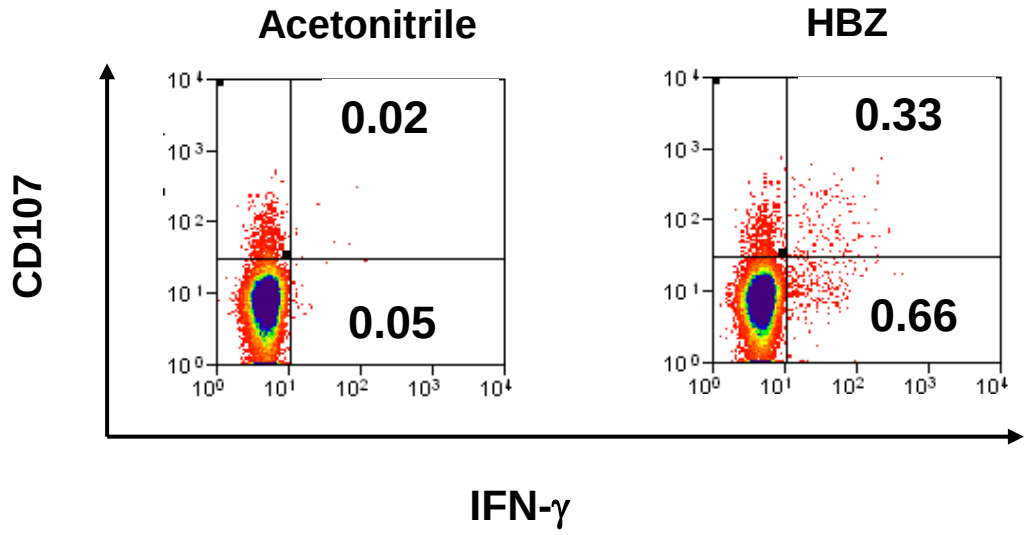
HBZ-specific CD8+ T cells exist & are functional

IFNg ELISpot





CD107a staining



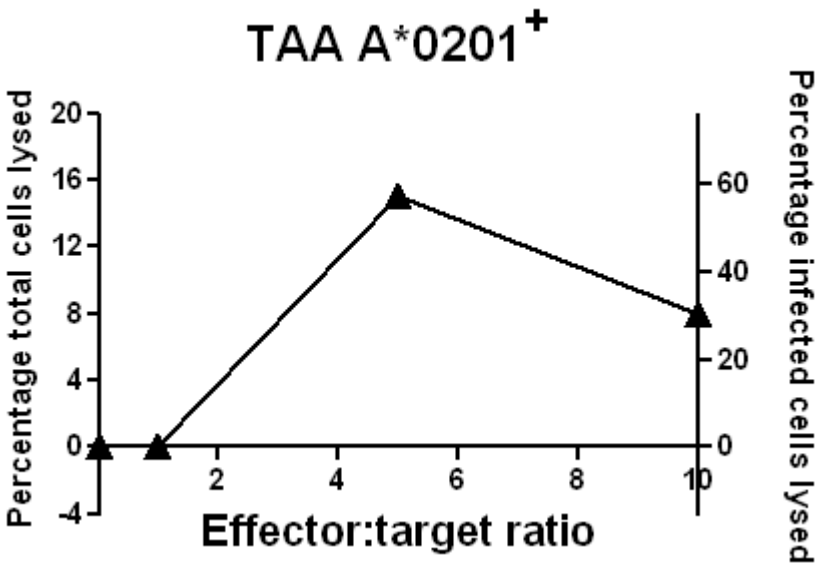
Aileen Rowan

Imperial College
London

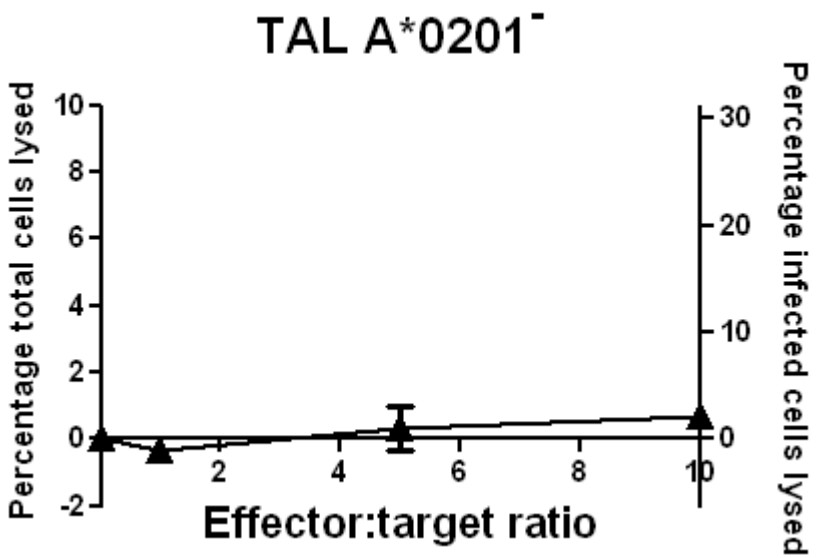
HBZ-specific CTL clone can kill infected cells

Chromium release

HLA matched



HLA mismatched



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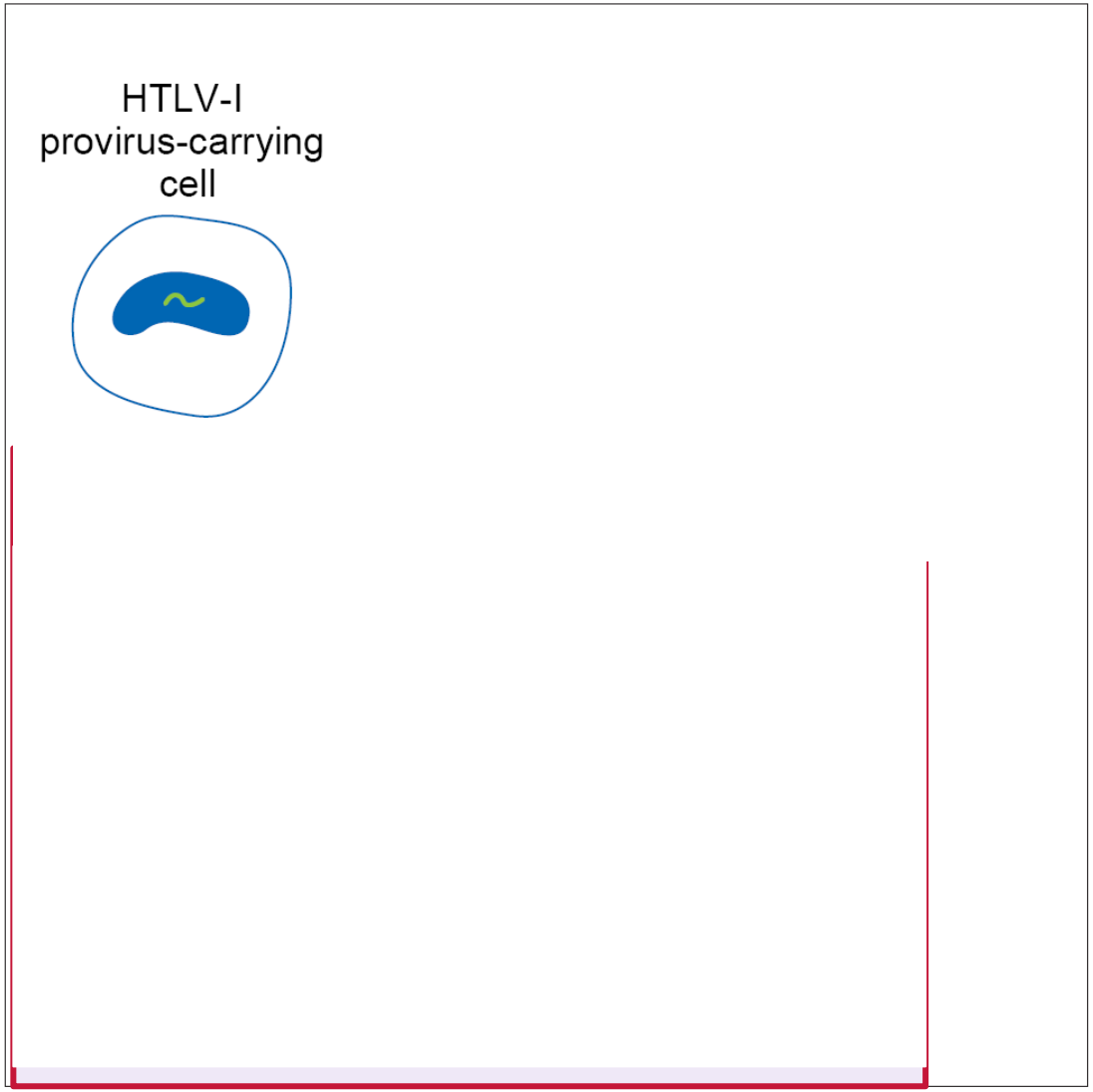
Why HBZ?



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Why HBZ?

HBZ inhibits expression of other HTLV-I genes

=> Evade immune response

HBZ expression drives infected cell proliferation

HBZ-specific CTL

Proviral load increases.

- In HTLV-1 specificity is an important determinant of $CD8^+$ T cell efficiency

- The most efficient responses are specific for HBZ

MacNamara et al **PLoS Pathogens** 2010

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Determinants of $CD8^+$ T Cell Efficiency

What makes a good HLA class I molecule?

- Specificity
- KIRs

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Background: KIRs

Killer Immunoglobulin-like Receptors

Activating & inhibitory isoforms

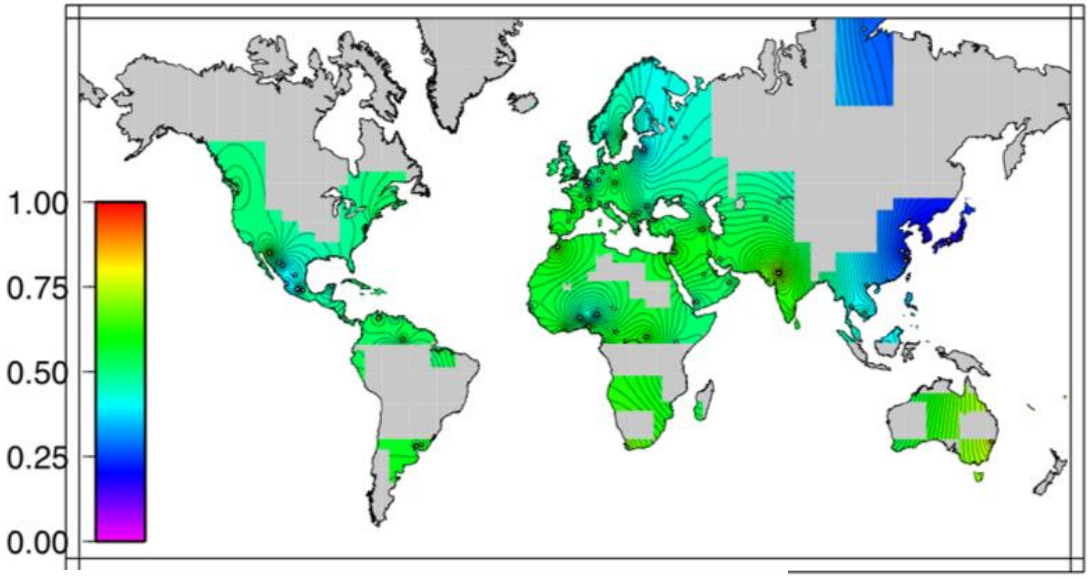
Expressed on NK cells & a subset of T cells

Bind HLA class I molecules

KIR2DL2

Inhibitory receptor

Binds HLA-C molecules



Hypothesis:

Killer immunoglobulin–like receptors (KIRs) influence HLA class I-mediated immunity

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HCV

Infects 170 million

- 30% Spontaneous viral clearance
- 70% Chronic infection

HLA-B57 protective

Cohort: N=702

HTLV-1

Infects 10-20 million

- 95% Asymptomatic carrier (AC)
- 1-2%HAM/TSP
- 2-5%Leukemia

A02, C08 protective

B54 detrimental

Cohort: N=432

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Method: immunogenetics

Hypothesis: KIR genotype determines efficiency of the HLA-restricted response

- Hepatitis C Virus (HCV) & Human T Lymphotropic Virus (HTLV)
- Known protective & detrimental HLA class I molecules
- Stratify cohort by KIR genotype
- Investigate how the protective/ detrimental effect of the HLA molecules is altered

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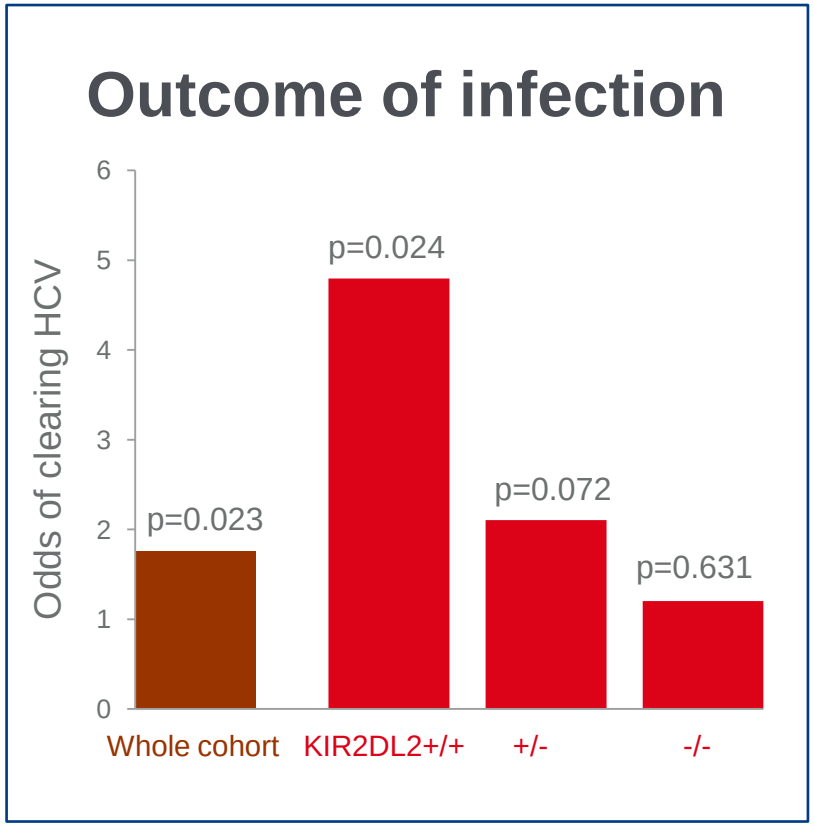
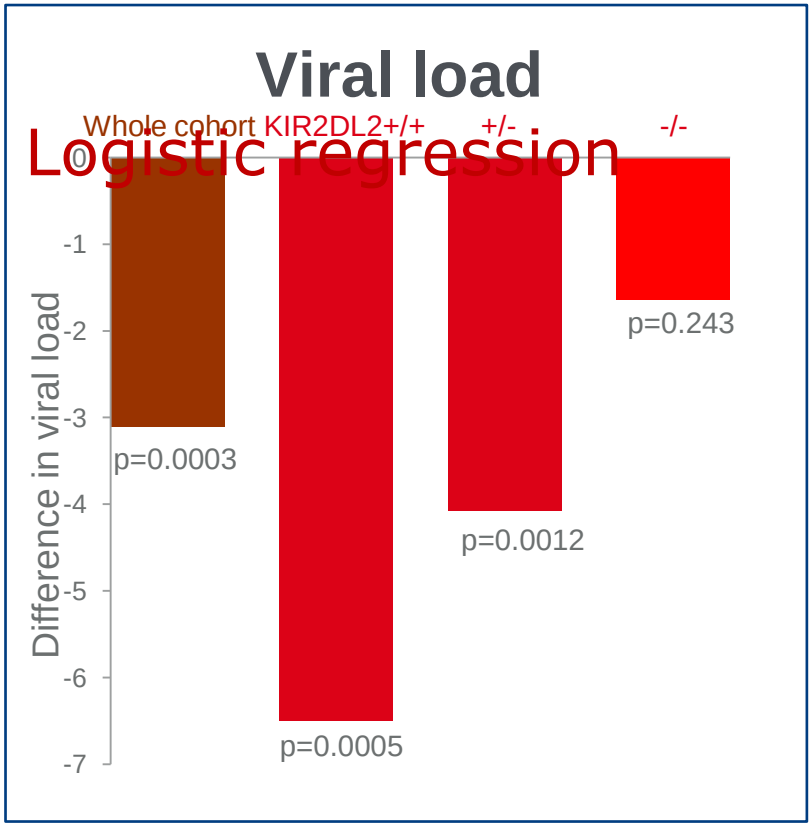
HCV:*KIR2DL2* enhances *B*57* protective effect





***KIR2DL2* enhancement strengthens with increasing copy number**

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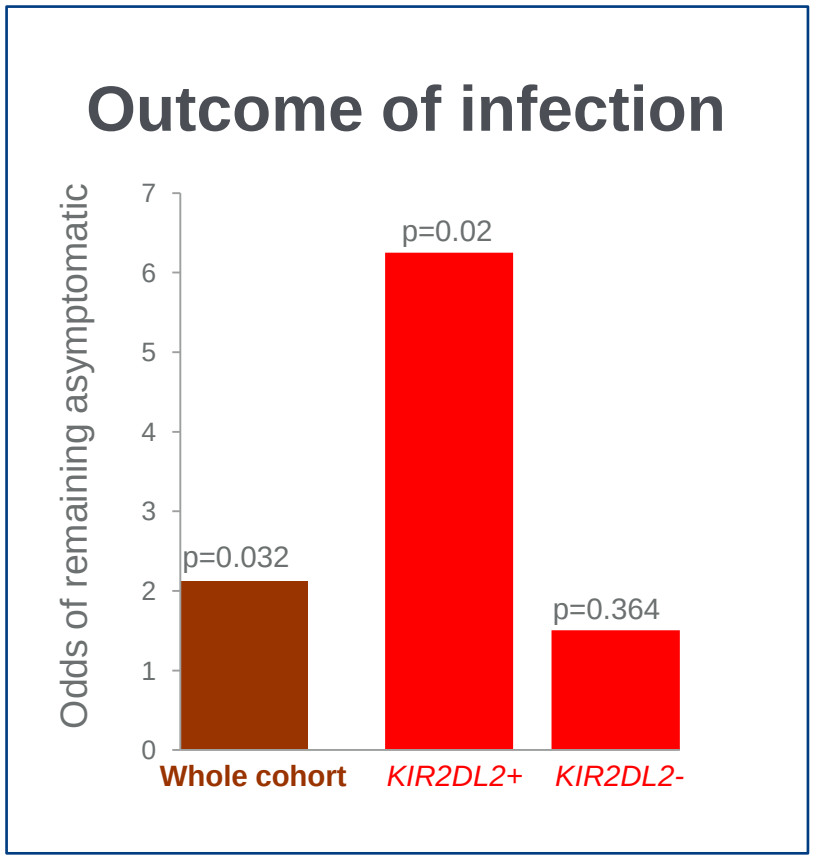
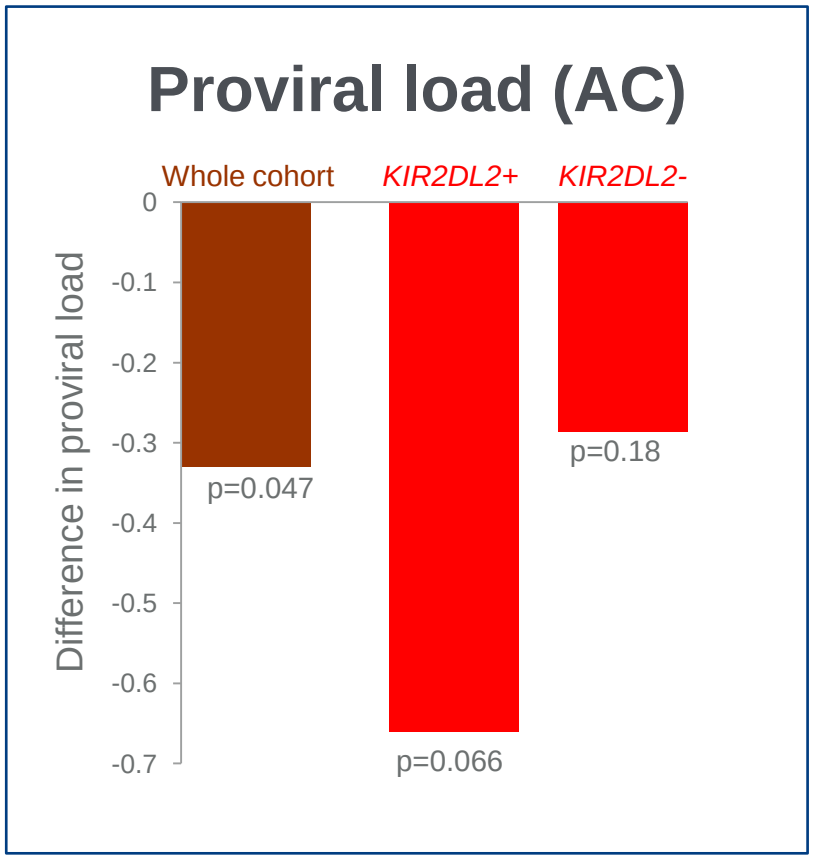
HTLV-1: *KIR2DL2* enhances protective effect of *C*08*





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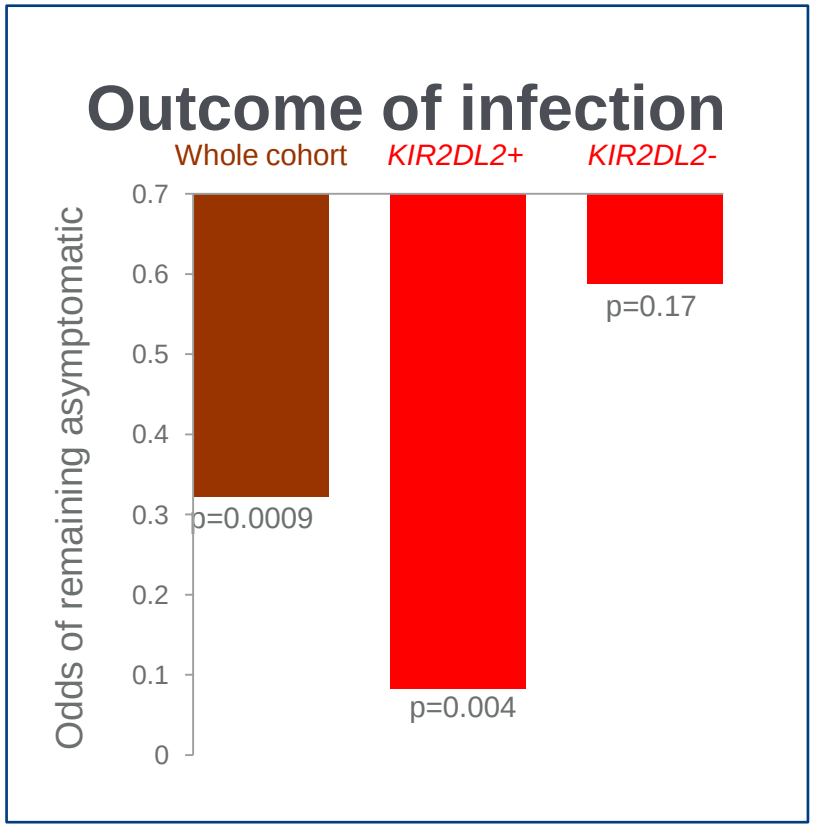
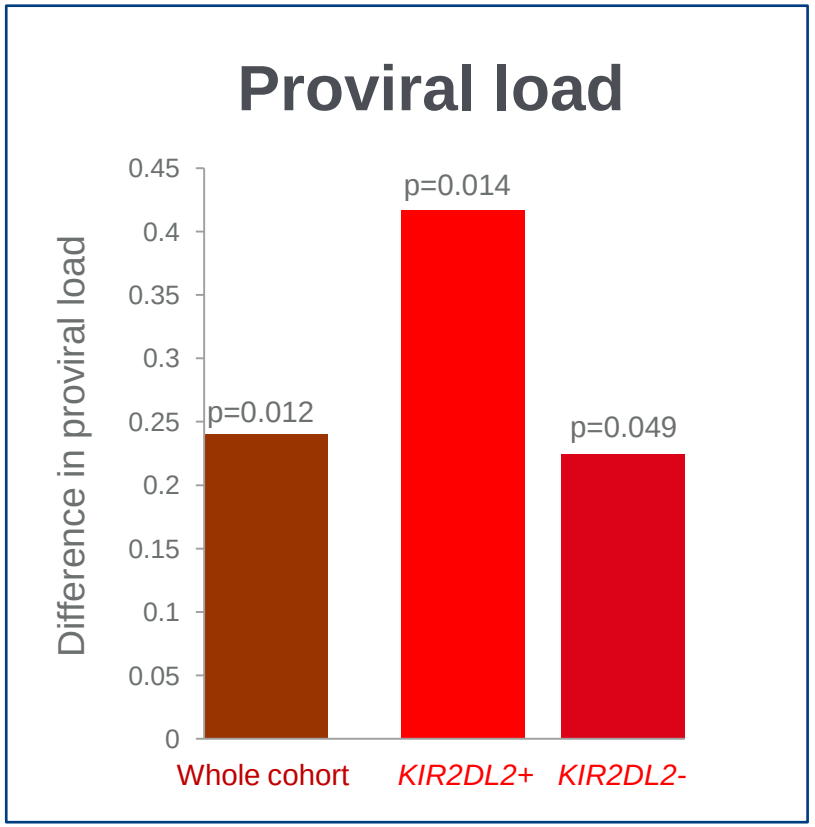
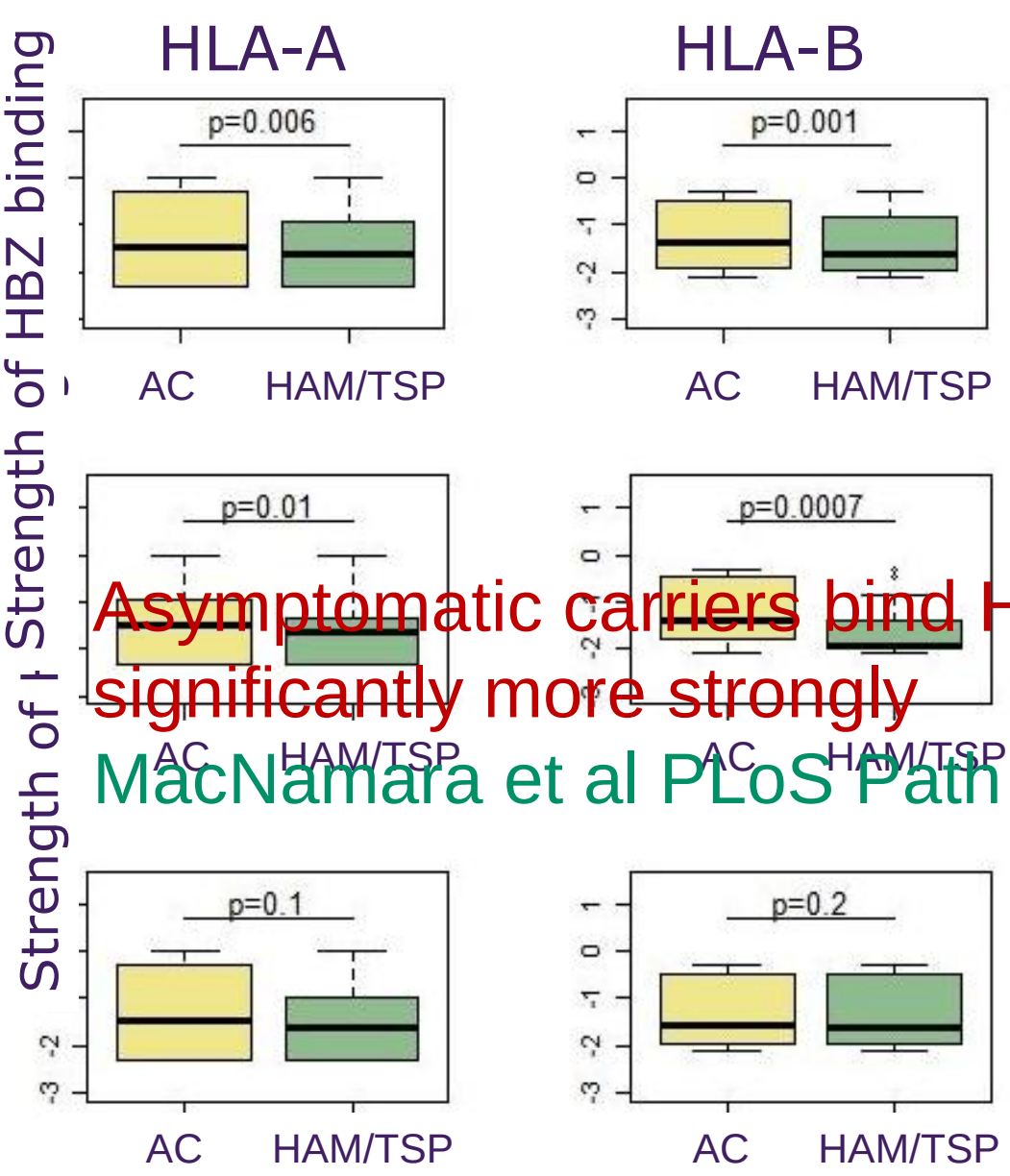
HTLV-1: *KIR2DL2* enhances detrimental effect of *B*54*







Whole cohort
P=0.00005

KIR2DL2+
P=0.00006

KIR2DL2-
P=0.06

Asymptomatic carriers bind HBZ significantly more strongly

MacNamara et al PLoS Path 2010

KIR2DL2 enhances protective effect of binding HBZ by multiple HLA class I molecules

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KIR2DL2: interim summary

KIR2DL2 enhances protective and detrimental HLA class I-mediated antiviral immunity

HTLV-1

- *C*08*: disease status
- *C*08*: proviral load
- *B*54*: disease status
- *B*54*: proviral load
- HBZ binding: disease status

HCV

- *B*57:* viral clearance
- *B*57 :*viral load

Explanation of why HLA don't always work

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Other KIR:HLA studies

- Khakoo *et al.* (2004) HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science
- Martin *et al.* (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nature Genetics
- Lopez-Vazquez *et al.* (2005) Protective effect of the HLA-Bw4I80 epitope and the KIR3DS1 gene against the development of hepatocellular carcinoma in patients with hepatitis C virus infection. J Infect Dis
- Martin *et al.* (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nature Genetics

Etc etc ...

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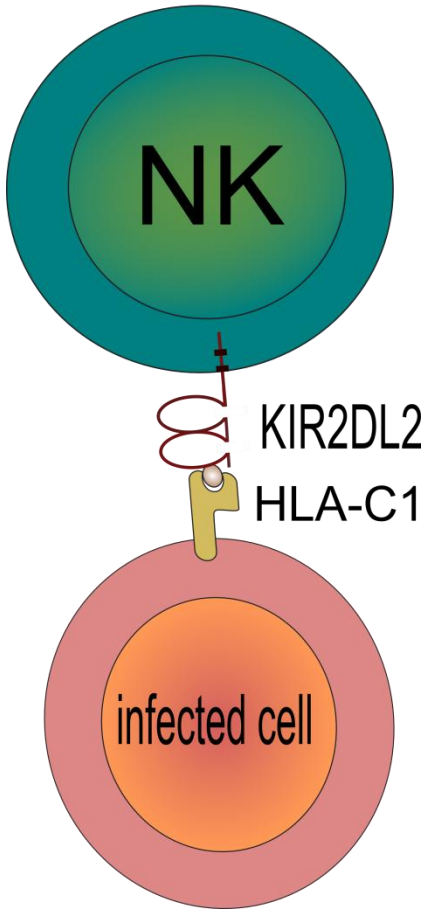
Direct NK killing of virus infected cell



Predict:

1) KIR with its ligand would have an effect on outcome
2) HLA class I molecules that do not bind the KIR would have no effect
3) Effect would be unidirectional (consistently protective or consistently detrimental

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Our observations quite different ...

Predict	Observe
KIR with its ligand would have an effect on outcome	
HLA class I molecules that do not bind the KIR would have no effect	
Effect would be unidirectional	

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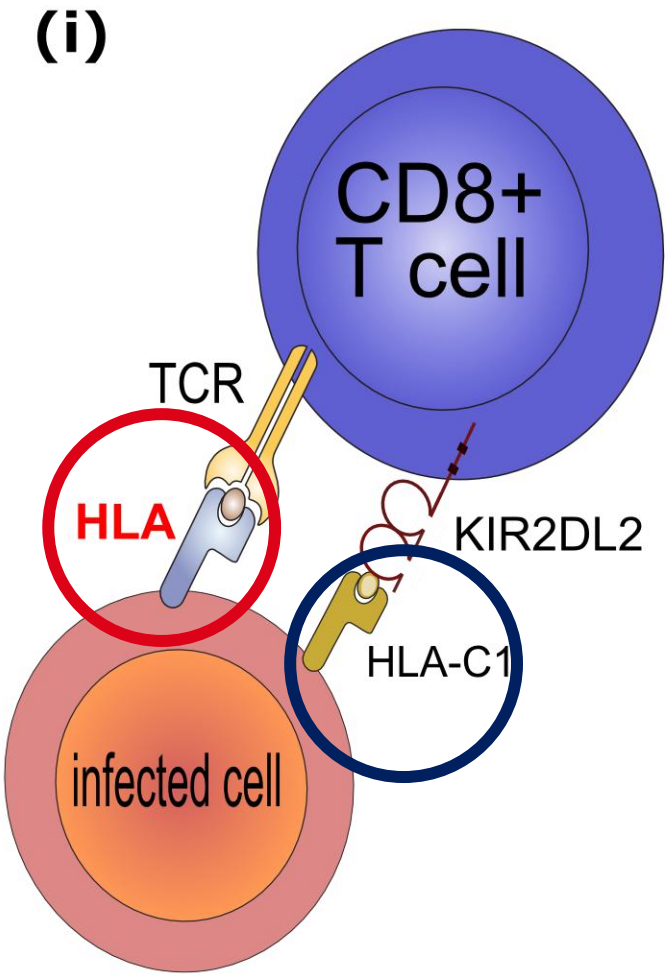
Postulated Mechanisms



Seich al Basatena et al PLoS Pathogens 2011

What next?

- How general is KIR2DL2-enhancement of adaptive immunity?
- What is the mechanism?
  - Does KIR2DL2 enhance the efficiency of $CD8^+$ T cells?
  - Does KIR2DL2 enhance the survival of $CD8^+$ T cells?

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Summary: Determinants of efficiency I

Used epitope prediction software to i… pr…

TAKE HOME 1

- General approach to investigate determinants of immune protection
- Protein specificity an important determinant of protection
- Immunogenicity is not

…d

HBZ
gag
p21
pol
p27
tax
rex
p30
p13
pro
p12
env

"worst"

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Summary: Determinants of efficiency II

TAKE HOME 2

- An "innate" receptor is enhancing HLA class I associations
- Potential explanation for incomplete penetrance of protective/ detrimental HLA traits
- Novel role for KIR in adaptive immunity?

Ongoing projects

Can non-lytic CD8+ T cell effector mechanisms select for viral escape variants?

How can we estimate diversity (viral clones, TCR, fish….)?

How can we understand KIR-associated polymorphisms in HIV-1?

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Can Keşmir
Keith Gould

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Links to Related papers

Epitope prediction:

T-cell epitope prediction: rescaling can mask biological variation between MHC molecules.

MacNamara A, Kadolsky U, Bangham CR, Asquith B. PLoS Comput Biol. 2009 Mar;5(3):e1000327.

HBZ:

HLA class I binding of HBZ determines outcome in HTLV-1 infection.

Macnamara A, Rowan A, Hilburn S, Kadolsky U, Fujiwara H, Suemori K, Yasukawa M, Taylor G, Bangham CR, Asquith B. PLoS Pathog. 2010 Sep 23;6(9):e1001117.

In vivo expression of human T-lymphotropic virus type 1 basic leucine-zipper protein generates specific CD8+ and CD4+ T-lymphocyte responses that correlate with clinical outcome.

Hilburn S, Rowan A, Demontis MA, MacNamara A, **Asquith B**, Bangham CR, Taylor GP.

J Infect Dis. 2011 Feb 15;203(4):529-36.

KIRs:

KIR2DL2 enhances protective and detrimental HLA class I-mediated immunity in chronic viral infection.

Seich Al Basatena NK, Macnamara A, Vine AM, Thio CL, Astemborski J, Usuku K, Osame M, Kirk GD, Donfield SM, Goedert JJ, Bangham CR, Carrington M, Khakoo SI, Asquith B. PLoS Pathog. 2011 Oct;7(10):e1002270.

In contrast to HIV, KIR3DS1 does not influence outcome in HTLV-1 retroviral infection.

O'Connor GM, Seich Al Basatena NK, Olavarria V, MacNamara A, Vine A, Ying Q, Hisada M, Galvão-Castro B, Asquith B, McVicar DW. Hum Immunol. 2012 Aug;73(8):783-7.

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Links to related papers

Some recent reviews:

HTLV-1: Persistence and pathogenesis.

Cook LB, Elemans M, Rowan AG, Asquith B.

Virology. 2013 Jan 5;435(1):131-40.

The efficiency of the human CD8+ T cell response: how should we quantify it, what determines it, and does it matter?

Elemans M, Seich Al Basatena NK, Asquith B.

PLoS Comput Biol. 2012;8(2):e1002381.