The impact of PIRCHE-II and Ischemia Reperfusion Injury on dynamics of CD4+ T memory cells in Liver Transplant recipients

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Abstract

Aim: PIRCHE-II represents HLA epitopes recognized by T cell indirect allorecognition and hypothesized to reflect potential CD4+ T cell alloreactivity between transplant pairs. However, the actual impact on recipient CD4+ T cell dynamics is still unclear. Furthermore, ischemia-reperfusion injury (IRI) during transplantation is known to augment CD4+ T cell alloreactivity. This study aims to identify CD4+ T cell subsets that correlate with PIRCHE-II in orthotopic liver transplant (OLT) recipients with and without IRI.

Methods: We studied 39 OLT recipients (21 IRI+ /18 IRI-), calculating PIRCHE-II from mismatched peptides presented by HLA-DRB1,3,4,5. No significant score differences were found between IRI+ and IRI- groups (p=0.19). Recipient PBMCs were collected at Preop, Early (1-4mo), and Late (6-12mo) post-OLT for CD4+ T cell immunophenotyping and assessment of alloreactive CD4+ T memory responses using activation induced markers and cytokine secretion assays with donor splenocytes. We analyzed correlations with PIRCHE-II in context of IRI data.

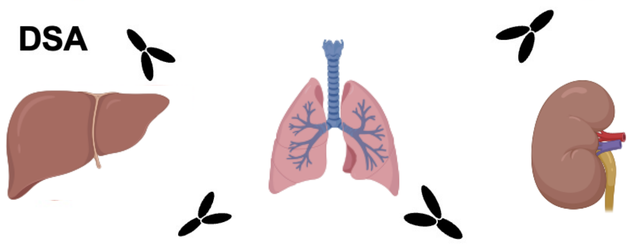
Results: Immunophenotyping at Late post-OLT showed that higher PIRCHE-II negatively correlated with frequencies of Naive CD4+ T cells (r=-0.31, p=0.05) and positively correlated with increased Effector Memory Tfh cells (r=0.29, p=0.07). A negative correlation was also exhibited between higher PIRCHE-II and decreased frequencies of Memory Treg cells from Pre to Late (r=-0.37, p=0.02). Within IRI+, negative correlations between PIRCHE-II were noted with Naive CD4+ T (r=-0.60, p<0.01) and Central Memory Tfh cells (r=-0.40, p=0.07), but positive correlations with Effector Memory T (r=0.40, p=0.07) and Memory Tfh17 cells (r=0.44, p=0.04) at Late. Increased PIRCHE-II negatively correlated with the change in frequencies of Memory Treg cells from Pre to Late in IRI+ only (r=-0.53, p=0.01).

Alloreactive CD4+ T memory responses in IRI+, showed a positive correlation between PIRCHE-II and the change in frequencies of Memory CD4+ T cells from Pre to Late (r=0.68, p<0.01). Conversely, in IRI-, a negative correlation was found with IL-10+ Memory CD4+ T cells at Late post-OLT (r=-0.44, p=0.09).

Summary: Memory CD4+ T cell subsets correlated with PIRCHE-II at the late post-transplant time point and were linked to IRI status. PIRCHE-II may indicate alloreactivity, useful for post-transplant management.

Background and Aim

Alloreactive humoral responses



- The development of Donor Specific anti-HLA Antibodies (DSA) is a major cause of allograft rejection after solid organ transplantation.
- DSA are developed due to HLA mismatches between donors and recipients.
- Quantifying HLA mismatches can enable the prediction of DSA development.

Evaluation of immunogenicity of HLA mismatches

HLA antigen/allele mismatches → Shift → HLA molecular mismatches

in vitro or in vivo assessment of the immunogenicity of each individual HLA mismatch combination

in silico algorithms may provide an alternative solution.

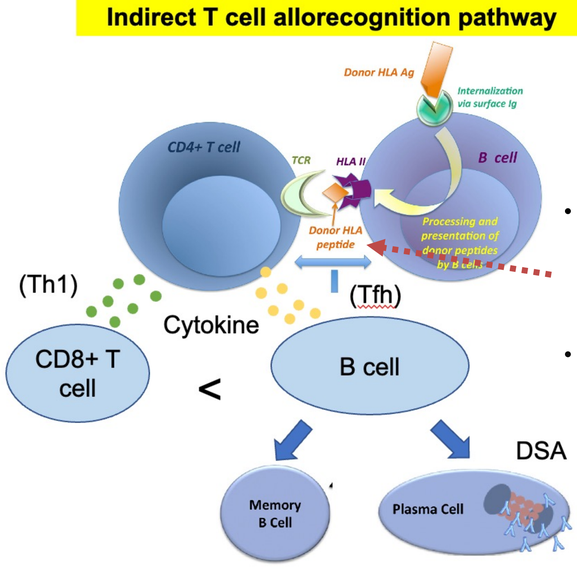
Current HLA allele database reporting more than 18,000 protein variants

HLAMatchmaker An Algorithm for Epitopes

PIRCHE match better.

- HLA mismatches at molecular level can now be quantified by *in silico* PIRCHE-II algorithm (**P**redicted **I**ndirectly **R**e**C**ognizable **H**LA **E**pitopes).

Indirect T cell allorecognition pathway



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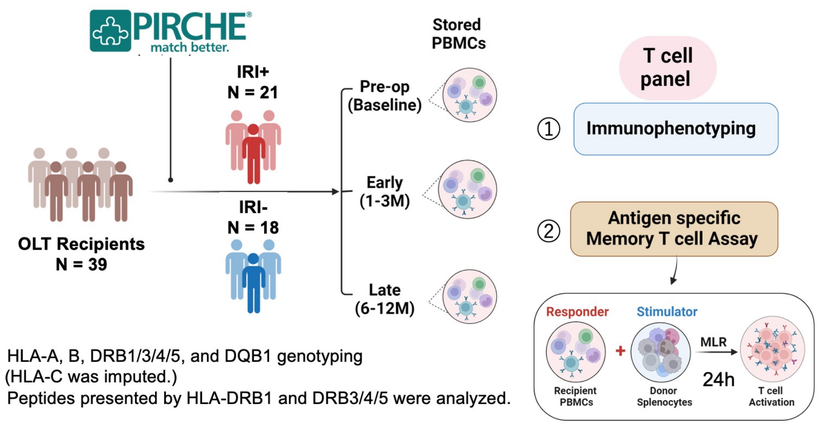
PIRCHE match better.

- PIRCHE-II predicts the number of HLA mismatch-derived peptides that can be presented by recipient HLA class II.
- These peptides promote CD4+ T cell responses.

- PIRCHE-II scores theoretically reflect the level of CD4+ T cell alloreactivity.
- However, actual impact on recipient CD4+ T cell dynamics is still unclear.

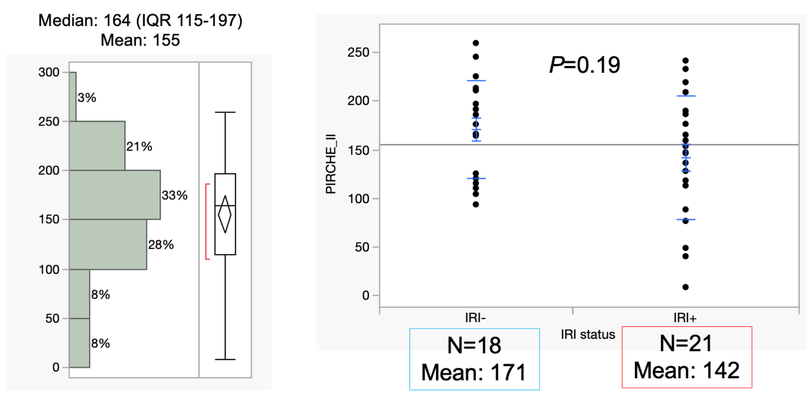
- Furthermore, ischemia-reperfusion injury (IRI) during transplantation is known to augment CD4+ T cell alloreactivity.
- This study aims to identify CD4+ T cell subsets that correlate with PIRCHE-II in orthotopic liver transplant (OLT) recipients with and without IRI.

Methods



Correlations with PIRCHE-II were analyzed in context of IRI data.

Distribution of PIRCHE-II (N=39)

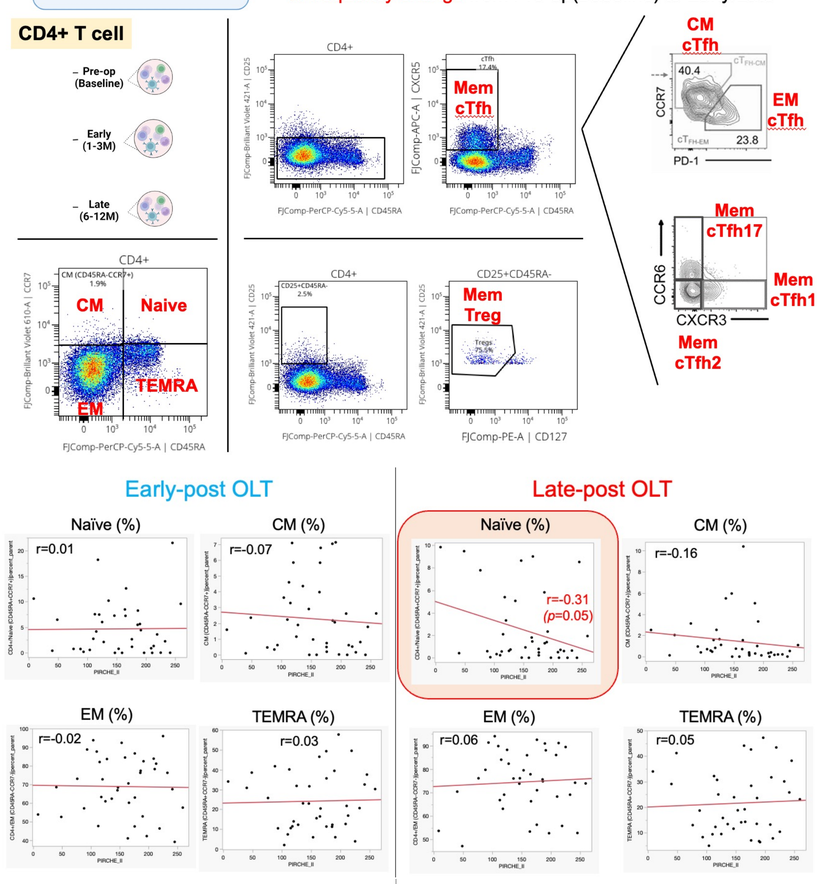


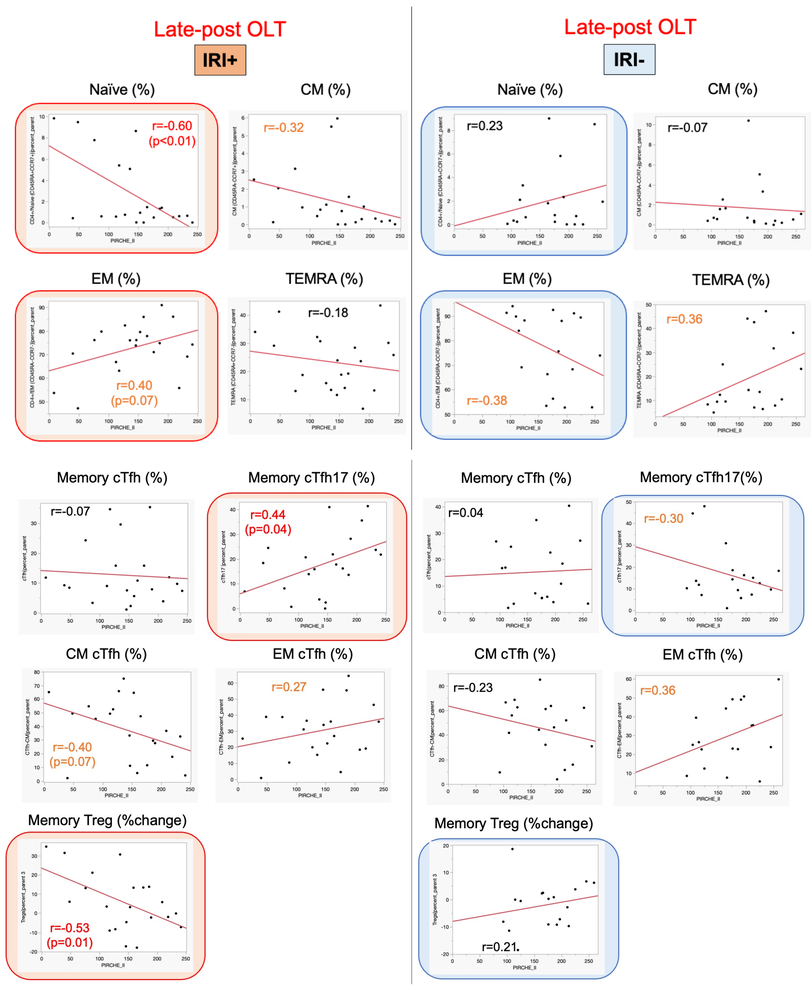
In the IRI- group, there are fewer individuals with low PIRCHE scores.

Results

① Immunophenotyping

- % at each (Early/Late) time point
- % frequency change from Pre-op(Baseline) to Early/Late



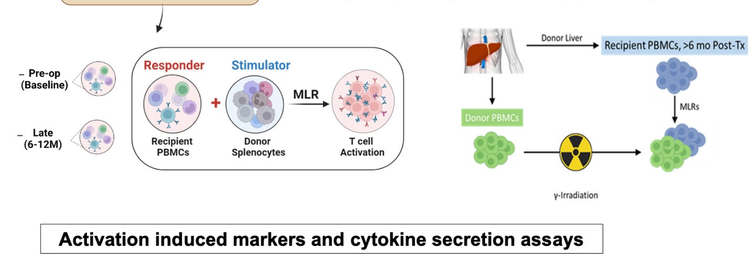


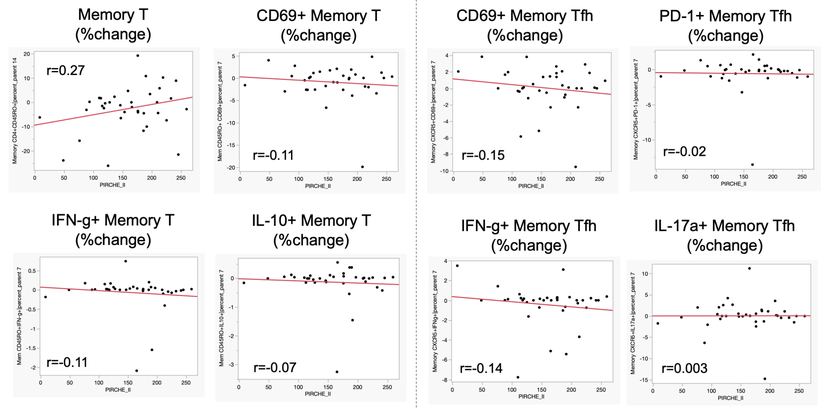
- It suggests that higher PIRCHE scores reflect higher alloreactivity.
  -The differentiation of Naive T cells progresses, leading to their decrease.
  -The differentiation from CM cTfh to EM cTfh appears to be advancing.
  -The reduction in Memory Treg cells is also a consistent finding.

- The IRI+ and the IRI- showed opposite correlations.
  -IRI promotes the alloreactivity, which may result in a more pronounced differentiation of Naive T cells, leading to their reduction, and an increased differentiation from CM to EM cells.

② Antigen specific Memory T cell Assay

- % at each (Late) time point
- % frequency change from Pre-op(Baseline) to Late

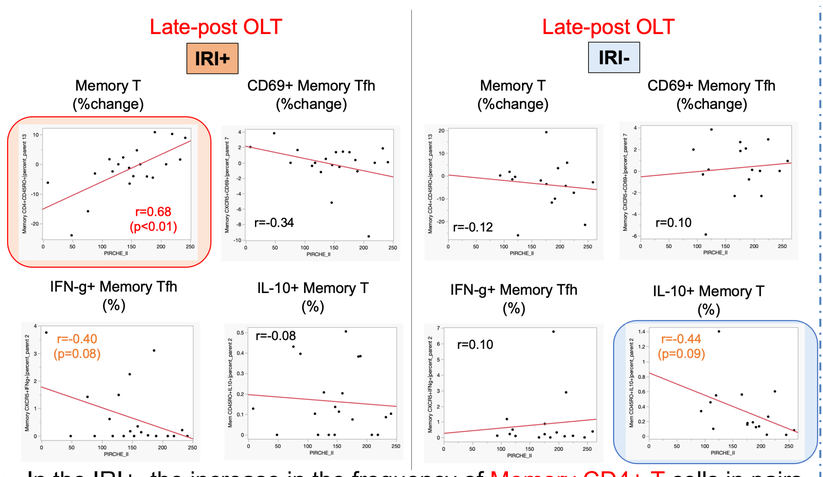




Activation induced markers and cytokine secretion assays

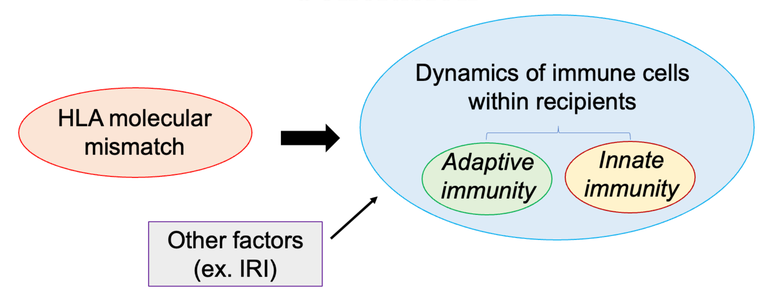
CD4+ T cell	Antigen-specific response	Activation	Effector/Function	Regulatory/Inhibitory
	Ki67 **CD45RO** **CXCR5(CD185)**	CD40L CD69 CD134 (OX40)	IFN-g	PD-1 IL-10 IL-2 IL-4 IL-17A

- **Memory CD4+ T cells (CD45RO+)**
- **Memory Tfh cells (CD45RO+ CXCR5+)**



- In the IRI+, the increase in the frequency of Memory CD4+ T cells in pairs with higher PIRCHE-II seems consistent.
- In the IRI-, pairs with higher PIRCHE-II had a lower frequency of IL-10-producing Memory CD4+ T cells, a regulatory marker, which also appears consistent.

Conclusion



❖ **Memory CD4+ T cell subsets correlated with PIRCHE-II** scores at later transplant time point (6-12mo), and were linked to IRI status.

❖ PIRCHE-II may need to be considered in combination with IRI.

❖ PIRCHE-II may serve as a marker for alloreactivity and could have implications for post-transplant monitoring and management.

David Geffen School of Medicine

NIH GRANTS & FUNDING NIH Central Resource for Grants and Funding Information

DISCLOSURE(S)

Authors have nothing to disclose.