Immune-Mediated Toxicities

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Financial Disclosures

I have the following financial relationship to disclose:
- consultant for Bristol Myers Squibb

I will not discuss off label use or investigational use in my presentation.
Outline

1- Immune related adverse events (irAE) clinical presentation overview

2- Challenges of mitigating irAEs

3- Proposed irAE mechanisms to-date

4- Defining key pillars for setting up irAE focused translational program

5- Pressing needs and gaps in the field
Mechanism of action: “re-establish” T cells ability to attack tumor tissue

Immune Checkpoint Inhibitors (ICIs): over 80 FDA-approved indications in cancer

Response rate between 12%-65% depending on cancer type

Immune Checkpoint Inhibitors (ICIs): How can we improve ICI therapeutic successes?

1- Trigger immune response in non-responders / cold tumors

2- Overcome therapeutic resistance

3- Predict and mitigate immune related adverse events (irAEs)

Mechanism of action: “re-establish” T cells ability to attack tumor tissue”

Immune Checkpoint Inhibitors (ICIs): How can we improve ICI therapeutic successes?

Mechanism of action: “re-establish” T cells ability to attack tumor tissue

Bending the Survival Curve

Champiat, Journal of Thoracic Oncology, 2014
Bending the Survival Curve

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (95% CI), months</th>
<th>HR (95% CI) v ipilimumab</th>
<th>P</th>
<th>HR (95% CI) v nivolumab</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab Plus</td>
<td>72.1 (38.2 to NR)</td>
<td>0.52 (0.43 to 0.64)</td>
<td>&lt;.0001</td>
<td>0.84 (0.67 to 1.04)</td>
<td>&lt;.0001</td>
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<tr>
<td>Ipi (n = 314)</td>
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<tr>
<td>Nivolumab</td>
<td>36.9 (28.2 to 58.7)</td>
<td>0.63 (0.52 to 0.76)</td>
<td>&lt;.0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ipi (n = 315)</td>
<td>19.9 (16.8 to 24.6)</td>
<td>—</td>
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</tbody>
</table>

Wolchok, JCO, 2022
Immune Checkpoint Inhibitors (ICIs): How can we improve ICI therapeutic successes?

Mechanism of action: “re-establish” T cells ability to attack tumor tissue”

Lifesaving potential ICI therapy is severely limited by immune related adverse events (irAEs)
Case 9-2020: A 64-Year-Old Man with Shortness of Breath, Cough, and Hypoxemia

Case 9-2020 overview

- 12/28/2015: Brain metastasis discovered (1.7 cm), Lung Lesion (2 cm)
- 1/11/2016: Brain metastasis resected, melanoma
- 3/9/2016: First Dose of Combination Immunotherapy
  - Ipilimumab 3 mg/kg (CTLA-4 inhibitor)
  - Nivolumab 2 mg/kg (PD-1 inhibitor)
- 3/30/2016: Second Dose of Combination Immunotherapy
- 4/4/2016: Admitted with Pneumonitis
- 5/12/2016: Admitted with Colitis
- 5/25/2016: Immune System Started to Attack Nerves
- 5/27/16: Intubated in intensive care
- 6/5/16: Expired
- Striking Findings at Autopsy:
  - Histological evaluation of lung nodule = necrosis
  - Neuropath Report = T cell (CD3) infiltrate causing sensory ganglionitis, loss of myelinated fibers, naked axons
  - Fungal Pneumonia/Fungal Colitis = Aspergillus Terreus

irAEs incidence will continue rising with ICI becoming standard of care

MHG treats over 1,000 patients/year with ICI

30% of US cancer patients eligible for ICIs

→ This is ONLY for monotherapy
→ Not accounting for combination & adjuvant therapy

Haslam, Jama Network Onc 2020
MGH inpatient irAE admissions

Chronic irAE are more prevalent than previously recognized

• chronic irAEs: defined as those persisting for >12 weeks after discontinuation of an anti-PD-1/PD-L1
• chronic irAEs occur in 43.2% of patients (ref. below)

• Why the lack of irAE recognition?
  → acute irAEs will at least improve with steroids and often resolve altogether
  
  → irAE reporting in early clinical trials focus on most frequent irAE (occur in ≥10% participants).
    Low-frequency events are under-reported/recognized regardless of their aggregate prevalence

  → initial clinical trials enrolled patients with metastatic cancer; characterizing chronic and long-term events is challenging because of limited life expectancy constraining long-term follow-up

  → presence of multiple co-morbidities in cancer patients can impair the identification of chronic irAEs

Most irAEs occur by **week 24** (6 months).

- **Combination** therapy **more toxic** than monotherapy.

- **Skin** most common.

- Toxicity **incidences vary** across **ICI used**.
  - Colitis and hypophysitis more common with anti-CTLA-4.
  - Thyroiditis more common with anti-PD1.

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**Kinetics of immune-related adverse events**

![Graph showing the kinetics of immune-related adverse events](image)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Toxicity Grade</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<tr>
<td>4</td>
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<td>6</td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
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</tbody>
</table>

- **Skin toxicity**
- **Liver toxicity**
- **Diarrhea, Hypophysitis**

The distribution, severity, and frequency of irAEs is related to the class of ICI used.
Are anti-CLTA-4 and anti-PD1/PDL1 irAE different?

- **Histopathologically**, anti-CTLA4 irAEs are **not distinguishable** from anti-PD1/PDL1 irAE

- **Treatment of irAEs** appears is **dependent** on the **organ involved** and the **severity** of the inflammation, rather than on the class of checkpoint inhibitor that was used
Limited understand of irAE mechanisms = limited tailored treatment solutions

- **Distinct** immunopathogenic **mechanisms** result in **irAEs**, leading to **distinct** histopathological **phenotypes** in each affected **organs**

- **Example**: immune-related nephritis or musculoskeletal adverse events can be:
  - lymphocyte driven
  - complement mediated
  - antibody mediated
  - caused by sterile inflammation (eg., pauci-immune glomerulonephritis) without any immune infiltrates on histopathological analysis

- Should these different presentations be **treated differently**?
  - Current guidelines **do not** make such a **distinction**.

Grade of Toxicity & General Guidelines for Management
Problem: everything is reactive NOT proactive

→poor understanding of irAE pathogenesis = we don’t know what are we treating

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Setting</th>
<th>Treatment/intervention</th>
<th>Immunotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild, asymptomatic</td>
<td>Outpatient</td>
<td>Observation</td>
<td>Continue</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Outpatient (close monitoring)</td>
<td><strong>Steroids</strong> (0.5-1 mg/kg/day), oral</td>
<td><strong>Hold</strong> pending resolution</td>
</tr>
<tr>
<td>3</td>
<td>Severe or medically significant</td>
<td>Inpatient</td>
<td><strong>Steroids</strong> (1-2 mg/kg/day), IV</td>
<td><strong>Hold</strong></td>
</tr>
<tr>
<td>4</td>
<td>Life threatening consequences</td>
<td>ICU</td>
<td><strong>Steroids</strong> (1-2 mg/kg/day), Consider additional agents (i.e. infliximab)</td>
<td><strong>Discontinue</strong></td>
</tr>
</tbody>
</table>

Is it important to avoid steroids? Impact on survival

- **Faje et al. Cancer 2018:** High-dose glucocorticoids for the treatment of ipilimumab-induced hypophysitis is associated with reduced survival in patients with melanoma. *Cancer 2018;124:3706-3714.* (referring to high dose steroids)

![Graph showing survival rates with and without steroids](image)

- **Metastatic melanoma treated with ipilimumab monotherapy complicated by hypophysitis**

<table>
<thead>
<tr>
<th>Months after starting ipilimumab</th>
<th>No. at risk:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LD</td>
</tr>
<tr>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>24</td>
<td>13</td>
</tr>
<tr>
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<td>11</td>
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<td>48</td>
<td>8</td>
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<tr>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>84</td>
<td>1</td>
</tr>
</tbody>
</table>

LD, median not reached, \( p = 0.002 \)
HD, median 23.3 months

Is it important to avoid steroids? Immunosuppression & efficacy


- Gourd E, et al. Lancer Oncol 2018: Baseline corticosteroids reduce activity of PD-L1 blockade *(referring to baseline steroids)*

- Bai X et al. Clin Cancer Res 2021: Early use of high-dose glucocorticoid for the management of irAE is associated with poorer survival in patients with advanced melanoma treated with anti-PD-1 monotherapy

→ The use of high dose steroids during PD1/PDL1 treatment can have potential detrimental effect on anti-cancer response

→ 947 patients, 509 (54%) developed irAEs

*Bai X et al. Clin Cancer Res. 2021 Nov 1;27(21):5993-6000*
Why study toxicities directly in patients?

→ Many complications not-well (yet) phenocopied in pre-clinical models
irAE presentation can phenocopy autoimmune disease presentation

- **Autoimmune disease**: evidence exists for T cell-mediated or B cell-mediated immunity against self-antigens, which is **not the case for all irAEs**.

- **Auto-inflammatory component** → activation of innate immune cells = primary form of immune dysregulation; little/no evidence for specific, self-antigen-directed adaptive immune responses

- **Auto-inflammation theory** → host-directed tissue inflammation at anatomical sites; local factors contributing to target organ damage

→ Let’s explore potential biological mechanism driving irAE
Breach of self-tolerance

- **T cells** play a central role in the immunopathogenesis of most irAEs.
- **T cells** are involved in numerous self-directed immune processes and can result from the loss of T cell tolerance.
- Presence of nascent autoreactive T and B cells having escaped central tolerance (inefficient purging) leads to pathogenic autoantibody formation.
- **Autoreactive T / B cells** might be generated during ICI therapy as a result of diversification and sub-compartmental expansion of lymphocytes.
Cytokine & chemokine production

- The release of inflammatory mediators from immune cells can lead to immune-mediated damage in tissues with an anatomic predisposition (e.g., interferon related signaling)
Cytokine & chemokine production

Circulating Cytokines Predict Immune-Related Toxicity in Melanoma Patients Receiving Anti-PD-1-Based Immunotherapy


Clin Cancer Res; 25(5) March 1, 2019

Exploratory Cohort: 98 melanoma patients on ICI

Exploratory Cohort: 49 melanoma patients on ICI

Experiment: 65 cytokines profiled before and during ICI therapy

Results:

1- **11 cytokines** associated with high grade irAE at baseline and during treatment

- G-CSF, GM-CSF, Fractalkine, FGF-2, IFNA2, IL12p70, IL1a, IL1B, IL1Ra, IL2, IL13

2- Created a toxicity score – CYTOX – that they validated in independent cohort

**Other studies** have highlighted the potential role of other cytokines, such as L17, IL6, TNF, IL-1β, IL-2, and GM-CSF, but not IL-6, IL-8, G-CSF, or MCP-1, IFN-γ, IL-8, CXCL9, CXCL10, CCL19, and CXCL11

**Limitation:** all studies remain small

**Need large validation cohort**

Potential antigenic targets in immune-related adverse events: specialized proteins (endocrine organs), tissue restricted protein, microbiome

Wang et al. Trends Cancer. 2023 Apr 26:S2405-8033(23)00058-4
Off-target effects

- Off-target effects of ICIs on non-haematopoietic cell expressing the target immune checkpoint ligand

- Hypophysitis secondary to anti-CTLA4 could be due to enhanced complement-mediated inflammation due to direct binding of an anti-CTLA4 antibody with CTLA-4 expressed on normal hypothalamic and pituitary tissue

- Does NOT explain hypophysitis in response to anti-PD-1 (rarer)
Cross-antigen reactivity

- Release of **host antigens** from **tumor cells** undergoing **cytotoxic attack** (e.g., vitiligo in melanoma patients on ICI)

- **Self-antigen** might be released when **non-transformed tissues** around tumor microenvironment are **damaged collaterally** by **immune** cells directed against tumor

- **Antigenic cross-presentation / bystander activation** can facilitate antigen presentation and autoimmunity
Cross-antigen reactivity

**Article**

**T cells specific for α-myosin drive immunotherapy-related myocarditis**

Margaret L. Axelrod, Wouter C. Meijers1,3, Elles M. Screewe1,3, Juan Qin1, Mary Grace Carroll', Xiaopeng Sun, Elie Tannous1, Yueli Zhang1, Ayaka Sugiyama1,4, Brandie C. Taylor1, Ann Hanna1, Shaoyi Zhang1, Kauansk Amanchel1, Warren Tai1, Jordan J. Wright1, Spencer G. We1, Susan R. Opaleni1, Abigail L. Toren1, Jeffrey C. Rathmell1,8, P. Brent Ferrell1, Elizabeth J. Phillips1, Simon MallI1,2, Douglas B. Johnson1, James P. Allison1,2, Javid J. Mostela1,4, Justin M. Balko1,4

https://doi.org/10.1038/s41586-022-05432-3

Received: 31 January 2022
Accepted: 7 October 2022
Published online: 16 November 2022

**Science Immunology**

**Autoreactive napsin A–specific T cells are enriched in lung tumors and inflammatory lung lesions during immune checkpoint blockade**

Fiamma Berner, David Bomze, Christa Lichtensteiger, Vincent Walter, Rebeka Niederer, Omar Hasan Ali, Nina Wyss, Jens Bauer, Lena Katharina Freudenmann, and Lukas Pfister

+24 authors Authors Info & Affiliations

**Science Translational Medicine**

**Epitope spreading toward wild-type melanocyte-lineage antigens rescues suboptimal immune checkpoint blockade responses**


+27 authors Authors Info & Affiliations
The enrichment of the *microbiome* with certain bacterial species can:

→ **protect** against or **induce** irAEs

→ **influences immune lineage** specifications towards pro-inflammatory or regulatory cell subtypes

→ **Regulate the production** of pro-inflammatory or anti-inflammatory cytokines
Many toxicities occur at barrier organs including the skin, gastrointestinal tract and liver, and lungs.

- irAE associated with barrier organs may suggest that the **antigenic targets** of the immune response may be the **commensal microbiome**
  - not proven

Wang et al. Trends Cancer. 2023 Apr 26:S2405-8033(23)00058-4
Microbiome shaping host immune response

Cohort: 77 patients with advanced melanoma treated with ICI with a high rate of any ≥grade 3 irAE

Experiment: profiled the blood, tumor and gut microbiome

Results:
1- toxicities associated with more diverse peripheral T-cell repertoire;
2- significantly higher abundance of Bacteroides intestinalis in patients with toxicity;
3- upregulation of mucosal IL-1β in patient samples of colitis and in pre-clinical models
Several studies have highlighted the potential role of tissue-resident memory T cells in mediating/contributing to irAE pathogenesis across different tissues.
Tissue-resident memory (T\textsubscript{RM}) cells

- T\textsubscript{RM} T cells are long lived memory effectors that make up a majority of T cells in most human organs
- Play an important role in tissue surveillance and recall responses to pathogens
- T\textsubscript{RM} remain in tissue to expression of retention molecules (e.g., CD103, CD69, CD49a)
- T\textsubscript{RM} express PD-1 and other inhibitory receptors that control their re-activation
- Example of potential cascade of events:
  - Checkpoint blocking antibodies $\rightarrow$ bind & re-invigorate T\textsubscript{RM}
  - T\textsubscript{RM} expand and can produce Th1 cytokines (e.g. IFNg, TNFa) and activate myeloid cells
  - Macrophages and DCs can produce CXCL9-11 that can recruit additional circulating T cells

Genetically predisposed individuals to irAEs

- Germline genetic factors are strong determinants of **immune homeostasis**

- Phenotypic **similarities** between **irAEs** and **autoimmunity** → shared genetic factors?

- Association between **toxicity and responses** in some cases → shared genetic factors

- **Limitation**: Majority of genetic association studies to-date have **small sample size** (<200 patients), and lump together all **irAE types**, limiting the chance of identifying true associations

Genetically predisposed individuals to irAEs – IL7

Cohort: 1,751 patients treated with ICI across 12 cancer types
High grade irAE: 259 cases, 1,375 controls
All irAEs: 339 cases, 1,412 controls

Results – 3 genome-wide significant results
→rs16906115 near IL7 was replicated in 3 independent studies
→IL7, a critical regulator of lymphocyte homeostasis
→Patients carrying the IL7 germline variant exhibited significantly increased lymphocyte stability after ICI initiation, which may be predictive of downstream irAEs and improved survival

Study design – to validate the IL7 association specifically

Cohort: 214 melanoma patients on ICI
Results – association with rs16906115 near IL7 was replicated
→Patients carrying the risk allele demonstrated:
- increased pre-treatment B cell IL7 expression;
- increased irAE risk;
- divergent immunoglobulin expression;
- more B cell receptor mutations;
- distinct ICB-induced CD8+ T cell subset responses;
- skewing of T cell clonality
Several different HLA alleles associated with irAEs across multiple small studies → larger studies are needed
Smoldering inflammation scenario

- E.g., Rheumatoid arthritis-like inflammation of joints
- off-target T cell activation that may wax and wane over time

Burnout / irreversible scenario

- E.g., Endocrinopathies (pancreas, thyroid gland)
- irreversible cellular damage precluding physiological recovery (e.g., destructions of hormone-secreting cells)
- Require permanent hormone replacement therapy

Possible frequencies of chronic immune-checkpoint inhibitor-induced toxicities

The exact risks of acute toxicities becoming chronic (defined as persisting for at least 12 weeks beyond treatment cessation) are currently unknown.

Per Centages expressed are the percentages of acute toxicities that become chronic (defined as those that persist for at least 12 weeks following ICI discontinuation).

Time course and potential importance of key issues throughout the course of treatment with immune-checkpoint inhibitors
Key challenge: de-coupling tumor-specific versus non-tumor triggers associated with irAEs

ADCC = antibody-dependent cellular cytotoxicity
Multiple mechanisms have been proposed for irAEs – complex picture

Cross-antigen reactivity
- Tumor antigen stimulates immune response
- Skin, pituitary gland

Off-target effects
- Breach of self-tolerance
- Increase level of pre-existing circulating autoantibodies
- Naive lymphocyte
- T cell diversification
- Autoactive T cells
- B cell diversification
- Autoantibody formation

Cytokine & chemokine production
- Innate immune cells
- Target cells
- Adaptive immune cells
- Tissue

Tissue resident memory cells

Genetic predisposition
- Likely other mechanisms involved given broad expression of PD-1 beyond T cells

Microbiome shaping
- Host immune response
- Pro-inflammatory lineage shifts
- Inflammatory cytokine production

Importance of pursuing translational effort to improve clinical care

• Understand spectrum of presentations would enable developing:

→ **Biomarkers**: identify patients at risk to (stop treatment b/f irAE become irreversible)

→ **Rapid diagnostic tools**: irAE presentations are hard to diagnose

→ **Better targeted therapies** to treat irAE while maintaining anti-tumor immunity
Key elements to succeed in solving irAEs assembled

1- **Infrastructure** and **expert knowledge** for patient identification

2- Gathering **experts** across division of Medicine for **developing best practice** for clinical care and optimal **phenotyping**

3- **Platform** for oncologist, medicine experts and scientists **to connect**

4- **Champions** and **infrastructure** for sample **collection** (inpatient / outpatient)

5- **Collecting** the right tissue samples at clinically **relevant time points**

6- Access to **optimal technologies** to test biological hypotheses
Gathering experts & champions across division of medicine: Severe Immunotheraphy Complication Service

51 members across 6 department and 10 divisions of Medicine

20 members actively bridging between clinical and laboratory work
A patient centric approach:
Overview of the champions and infrastructure for sample collection

Leveraging MGH unique multi-disciplinary environment to empower our bedside-bench-bedside SIC translational research program

- **SIC Service Team & Oncology**
  - Nursing team
  - Clinical research coordinators
  - Administrative support

- **Surgery team IR team**

- **Translational Team**

- **Clinician Specialist**

- **Melanoma Biobank Group Rapid Autopsy**
### MGH patient cohort across irAE types (n=306):

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Colitis</td>
<td>N=101</td>
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</tr>
<tr>
<td>Arthritis</td>
<td>N=56</td>
<td>N=45</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>N=43</td>
<td>N=11</td>
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<tr>
<td>T1DM</td>
<td>N=12</td>
<td></td>
</tr>
</tbody>
</table>

**Cases**

- Patients on anti-PD-1 and/or anti-CTLA-4 with **histologically proven** irAEs

**Controls**

- Patients on anti-PD-1 and/or anti-CTLA-4 without irAEs

→ **1873 samples biobanked:** biopsies, blood, serum, plasma, body fluids, autopsy specimens
Solving irAEs requires collecting the right tissue samples at clinically relevant time points: exploration of scale, time, and modalities

Different scales

- ORGAN NETWORKS
- CELLULAR NETWORKS
- MOLECULAR NETWORKS
- GENES
ICI-associated myocarditis is highly morbid

- ICI-Myocarditis occurs in ~1% of patients but is often fatal in up to 40% of cases

- Major adverse cardiac events (MACE)
  - including arrhythmias, sudden cardiac death, heart failure, or cardiogenic shock
  - occur in ~25-50%

- Diagnosed by MRI or a biopsy showing T-cell infiltrates

Wang DY, Johnson DB et al. JAMA Oncol, 2018
irMyocarditis Study Design

Heart tissue:
Control heart tissue (n=8)
irMyocarditis (n=13)
7 PD-1/PD-L1 inhibitors
6 PD-1/CTLA-4 blockade
8 tumor histologies

Paired tumor tissue (n=4)

Blood specimens (n = 26)
Matched to heart
All pre-steroid samples

Heart single-cell RNA + TCRseq
(n=84,576 cells)

Immuno-histochemistry

Blood single-cell RNAseq+ TCRseq
71 secreted factors
(n=232,929 cells)
(Luminex)
Approach Overview

Control heart and blood
- No ICI exposure
- On-ICI, no myocarditis

Myocarditis heart and blood
- Dx by MRI or histology

Compare cell populations
Compare gene expression
Compare TCR repertoire
Compare circulating factors
1. What cellular and molecular perturbations define irAEs? (tissue ecosystem)

**Strategy: Single-cell genomic dissection of tissue lesions**

1. Biopsy collection
2. Single-cell sequencing
3. Distinct profiles

- scRNAseq
- *single cell TCR & BCR CITEseq, ATACseq

**Questions:**

- Solid Tissue
  - Dissociated Tissue
  - 1. Biopsy collection
  - 2. Single-cell sequencing
  - 3. Distinct profiles

**Strategy:**
- Single-cell RNA sequencing
- Cell populations in “control” patients
- Cell populations in “disease” patients
1. What cellular and molecular perturbations define irAEs? (tissue ecosystem)

**Strategy:** Single-cell genomic dissection of tissue lesions

1. Biopsy collection
2. Single-cell sequencing
3. Distinct profiles

scRNAseq
*single cell TCR & BCR
CITEseq, ATACseq

**Analyses:** mapping cell types, states, regulatory programs and neighborhoods of cells

“Control” ↔ “Disease”

Cell type ◦ Cell state spectrum

Inferring biology

In situ cell neighbors
Transcriptional program
Question: Which cell populations are more abundant in irMyocarditis?

- T cells, myeloid cells, dendritic cells, fibroblasts all shift in abundance

T cell subsets spanning effector and exhaustion programs enriched in cases

6 T/NK CELL SUBSET IN HEART

CD8 T2: CCL5, CXCL13
CD4 T1: IL7R, LTB
CD8 T1: CD27, LAG3
CD8 T4: STMN1, TOP2A
NK: KLRF1, KLRB1
Plasma cells: JCHAIN
CD8 T3: KLRG1, CX3CR1

T ABUNDANCE ANALYSIS

P = 5.12E-05
P = 0.009
P = 0.034
P = 0.050
P = 0.074
P = 0.102
P = 0.123
P = 0.767

10 broad cellular populations identified by

→ Should not be any T cells in the heart
→ Are these the same T cells fighting the tumor?
2. Defining phenotypic spectrum of T-cell clones in irAE lesions, blood, and tumor

Strategy: TCR analyses in paired samples

Analyses: deriving principles across tissues and irAEs

Questions:
How does TCR repertoire diversity correlate with irAEs and with response to treatment?
Can we identify the same TCR clones in tumor, blood, and irAE tissue lesions?
Are T cells recognizing the same antigens?
Can we identify shared T-cell receptor clones between heart and tumor? Shared TCR clones could help identify (1) important biology or (2) non-invasive biomarkers.

TCRs) serve as barcodes for T cells in heart or tissue

Autopsy cases (n = 4)

Inflammation in myocarditis tissue

Microdissection strategy to analyze ONLY affected tissue

Tumor invading normal parenchyma

T-Cell Receptor (TCR)
Question: Can we identify share TCR clones between paired heart and tumor?

Most expanded TCR clones in tumors and heart are NOT shared, suggesting distinct biology between anti-tumor immune response and irMyocarditis.
3. Does blood mirror tissue? Identifying biomarkers for patients at higher risk for irAEs

**Strategy: secreted factor monitoring and blood analysis**

**Analyses #1: can blood mirror tissue?**

- Immunophenotyping, single-cell RNAseq analyses (scRNAseq+TCR+BCR+204 proteins)

**Biomarkers could be:**
- particular TCR /BCR clone
- cell subset
- gene signature
- secreted factors
- cell surface protein marker
- germline susceptibility variant

**Analyses #2: cytokines and autoantibodies levels driving break of immune tolerance**

- Cytokines
- Activated cells

**Levels**

- Different scales
- Different measurement

**Time course**
Question: Can we identify share TCR clones between paired heart and blood?

Yes! TCR sharing between heart and blood, with fatality association

Blood CD8+T/NK Cell UMAP: 13 subsets, 75,480 Cells

No TCR sharing

Fatal irMyocarditis TCR sharing is distinct

Cluster 12: Cycling, CXCR3^hi CD8 T cells

Red = TCR clones found in heart and blood

13 subject IDs

MKI67

Increased Expression

CXCR3
Question: Are there secreted factors associated with ICI-myocarditis?

CXCR3 ligands and other factors involved in T cell recruitment elevated in patient blood

Can these factors predict irMyocarditis? Are these factors also associated with anti-tumor response?
Working towards unraveling the underpinnings of irAEs and identifying drug targets through 3 complementary strategies

(1) What are the cell populations & transcriptional programs enriched in the hearts of myocarditis patients?

(2) Are T cell clones shared between tumor, heart and blood?

(3) Can we identify circulating biomarkers associated with myocarditis and outcome?

- Immunophenotyping, secreted factors, single-cell RNAseq analyses (scRNAseq+TCR+BCR+204 proteins)
Overview of different ongoing single-cell multi-omics' efforts across organ systems

- **Myocarditis**
  (Daniel Zlotoff, Steven Blum, Neal Smith, Swetha Ramesh, Isabela Kernin)

- **Colitis**
  (Molly Thomas & Kamil Slowikowski)

- **Hepatitis**
  (Molly Thomas, Tos Chan, Neal Smith)

- **Arthritis**
  (Mazen Nasrallah & Gary Reynolds)

- **Neurotoxicity**
  (Hoang Tran & Amanda Guidon)

- **Nephritis**
  (Meghan Sise, Isabela Kernin)

- **Endocrinopathies**
  (Michelle Rengarajan, Rachelly Normand)
Where do we go from here?

irAE meta-analysis to guide follow-up studies:

- Are all irAE presentations created equal biologically?
- What are the shared and distinct populations/pathways involved?
- Which biological programs are distinct from tumor response?
- Should irAE all be therapeutically modulated the same way?
Working towards unraveling irAE underpinnings and identifying drug targets through multi-modal approaches

- DNA mutations and modification (TCR repertoire)
- Tissue organization & cell-cell comm.
- Detailed clinical variables
- Comparison between tissues from same indiv.
- Function and mechanisms
- Secreted factors Ligand-receptor
- Epigenomics of infiltrating cells
- Mapping cell activation states
- Prediction model
  - Culprit cell types
  - Activated molecular circuitry
  - Disease diagnosis and prognosis
  - Treatment response
Pressing needs and gaps in the field of irAE biomarker & mechanisms discovery

- **Larger irAE cohorts needed** – cross institutional collaborations required for gathering enough samples to identify biomarkers for rarer irAE presentations

- **Serial time point collection** – tracking factors over time to define biomarkers (e.g., pre-ICI, post-ICI/pre-irAE, at irAE diagnosis pre-steroid, post-steroid)

- **Distinguishing signature** of ICI-treatment response vs. irAE presentations

- **Developing consensus annotations** – irAE presentation & ICI-treatment response

- **ICI controls** – Analyzing the right types of controls to define irAE biomarkers (e.g., matching demographics, tumor, treatment, time points, no irAE)

- **Funding** – Currently few opportunities to study the underpinnings of irAE in patients
There is a critical need to work together to solve irAE & improve patient care
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The patients & families

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Always looking for talented trainees and postdocs!