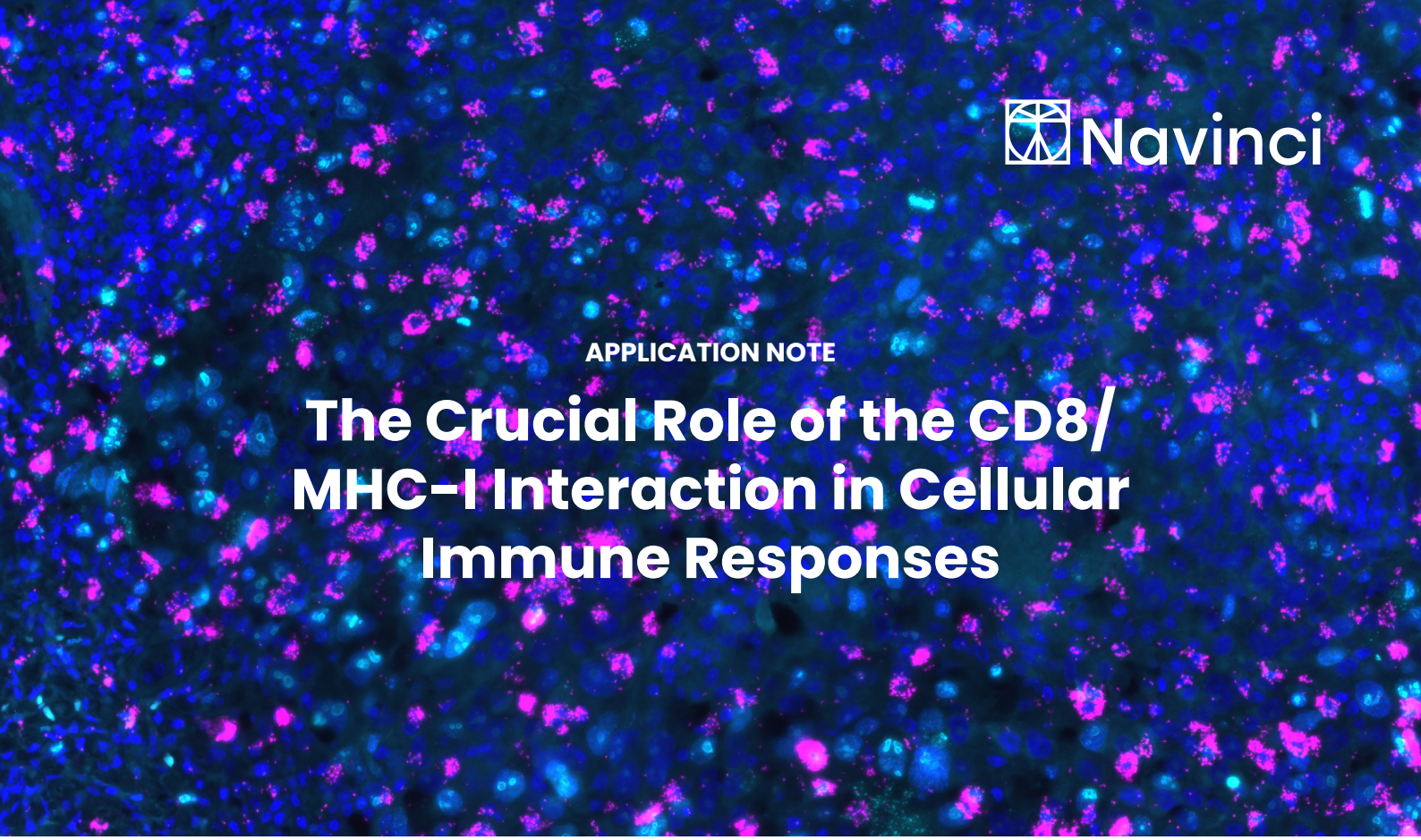


Navinci

APPLICATION NOTE

The Crucial Role of the CD8/ MHC-I Interaction in Cellular Immune Responses

Background

The interaction between the CD8 co-receptor and the major histocompatibility complex class I (MHC-I) proteins plays a pivotal role in orchestrating cellular immune responses. It is fundamental for the activation and regulation of cytotoxic T lymphocytes (CTLs) – key effectors in the adaptive immune system, tasked with the recognition and elimination of infected or abnormal cells. The interaction between CD8 and MHC-I not only ensures specificity in target cell recognition, but also modulates the strength and duration of immune responses.

In addition, the CD8/MHC-I interaction also contributes to immune response regulation. The affinity of CD8 for MHC-I is finely tuned, allowing for discrimination between normal and aberrant cells. This discriminatory capacity helps prevent autoimmune responses and maintain immune homeostasis.

Implications in Disease

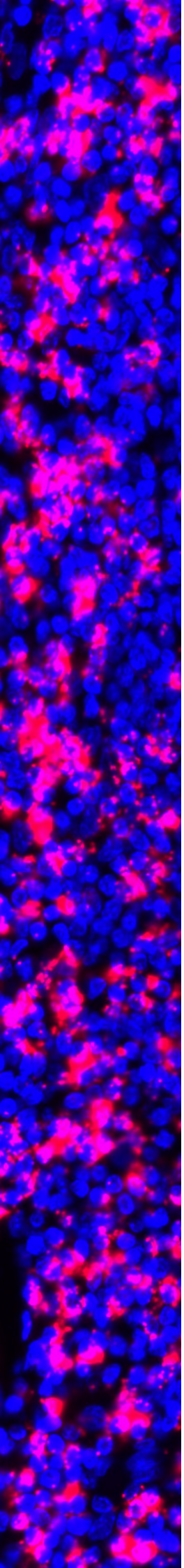
Alterations in the CD8/MHC-I interaction have been associated with various pathological conditions. For instance, **viral evasion strategies** often involve interference with MHC-I presentation or downregulation of CD8 expression, compromising the ability of CTLs to recognize and eliminate infected cells (Hewitt 2003).

Furthermore, the interaction plays a critical role in **cancer immunosurveillance** due to the cytotoxic function of CTLs against tumor cells (Burnet 1970). Thus, MHC-I downregulation or alterations in peptide presentation can hinder effective antitumor immune responses, enabling tumor escape from immune surveillance.

In **autoimmune diseases**, dysregulation of the CD8/MHC-I interaction may contribute to the activation of autoreactive CD8+ T cells, resulting in tissue damage and inflammation (Walter and Santamaria 2005). Furthermore, some pathogens share molecular similarities with host antigens, leading to molecular mimicry and cross-reactivity, and contributing to the initiation/ exacerbation of autoimmune diseases.

Therapeutic Strategies

Therapies such as immune checkpoint inhibition aim to enhance the CD8-mediated immune



response against cancer by blocking inhibitory signals (Pai et al. 2023). Adoptive T cell therapies leverage the cytotoxic potential of CD8+ T cells, emphasizing the importance of the CD8/MHC-I interaction in targeting cancer cells (Chan et al. 2021).

Modulating the CD8/MHC-I interaction is also a potential avenue for autoimmune disease treatment. Therapies that target specific steps in the CD8-mediated immune response, such as blocking co-stimulatory signals or altering MHC-I presentation, are under investigation for their potential to modulate autoimmune responses (Yang, Halima, and Chan 2023). Understanding the dynamics of the CD8/MHC-I interaction is therefore essential for developing strategies to enhance immune responses in the context of infections, cancer, and autoimmune diseases.

To this end, methods for uncovering protein-protein interactions (PPIs), as opposed to merely observing protein expression, are instrumental in illuminating protein function and functional aberrations. The possibility to observe the dynamic formation and disassembly of protein complexes has multiple applications. Among others, it helps predict the response to drug treatments, stimulation/inhibition, and elucidates crosstalk between signaling pathways and cell-cell communication via surface proteins. Such information, contextualized by the precise location of the PPI in cells and tissues, sheds light on the spatiotemporal communication between proteins in their native milieu. This can be achieved by employing the Naveni® *in situ* proximity ligation technology, which acts as a molecular ruler and creates a signal if and only if two target proteins are within an interaction range of 10-40 nm (Fig. 1).

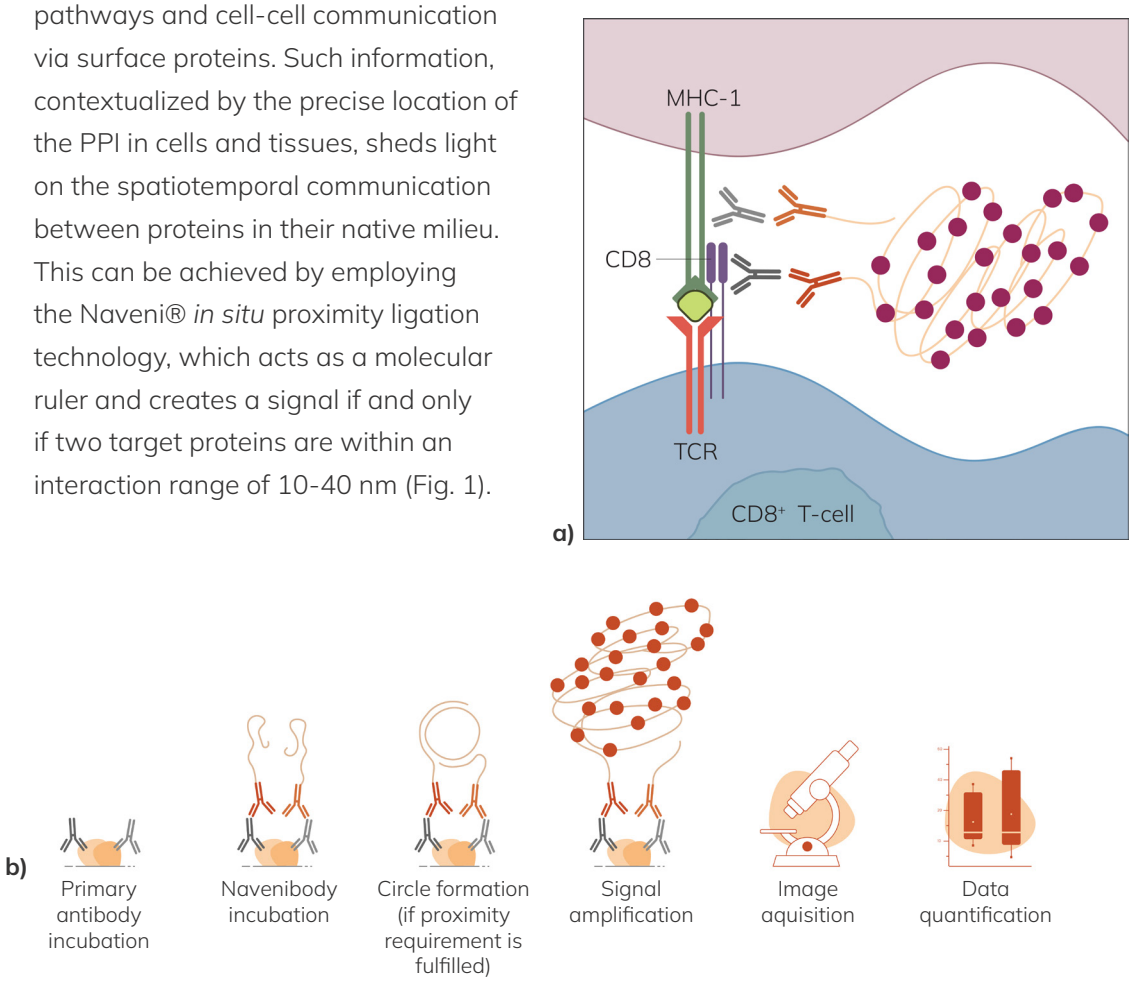


Fig. 1. a) Schematic representation of the CD8/MHC-I interaction and how it is detected by primary antibodies and Navenibodies (orange) included in the Naveni® CD8/MHC-I kit. b) Assay workflow.

To illustrate the priming of cytotoxic T cells, the Naveni® CD8/MHC-I Atto647N kit was used to detect the interaction in tonsil, where such activation is to be expected (Fig. 2). Technical negative controls were performed by replacing one of the primary antibodies – rabbit anti-CD8 or mouse anti-MHC-I – with the respective isotype control. Clear interaction staining was observed at the edge of the germinal centers (image to the right), while the negative controls were blank.

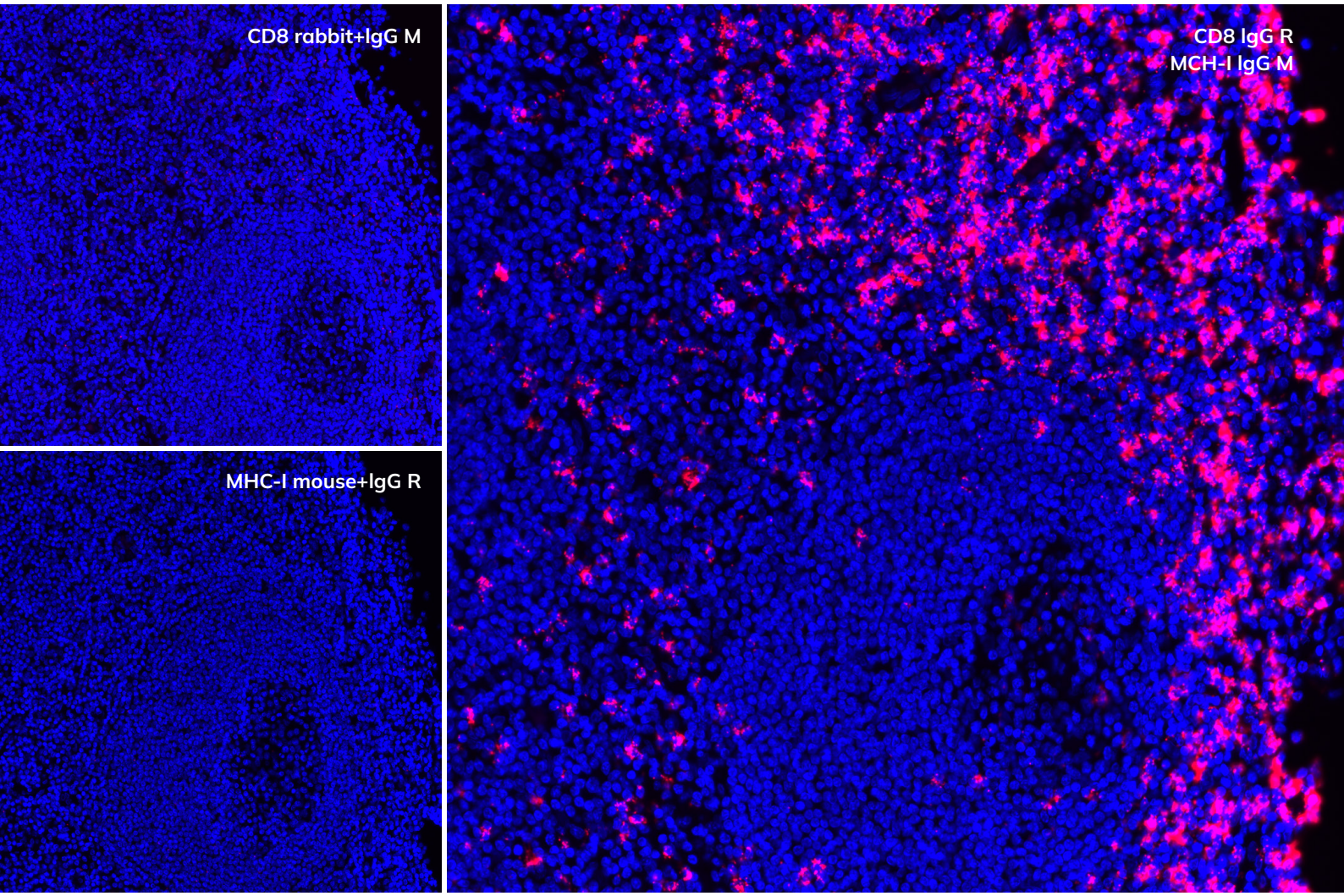


Fig. 2. FFPE tonsil showing positive staining for the CD8/MHC-I interaction and no staining in technical negative controls with the Naveni® CD8/MHC-I kit.

The interaction was further explored in Hodgkin lymphoma and hepatocellular carcinoma together with immunofluorescence co-stainings (Fig. 3.). CD3 was used for visualization of T cells in the tumor microenvironment. Ki-67 was used as a proliferation marker. It is usually highly expressed in malignant cells but is rare in normal ones. High levels of adjacent co-expression between the Naveni® signal and the immunofluorescent stains were observed in both cancer tissues, which may indicate hot tumors and poor prognosis (in the case of the liver cancer sample).

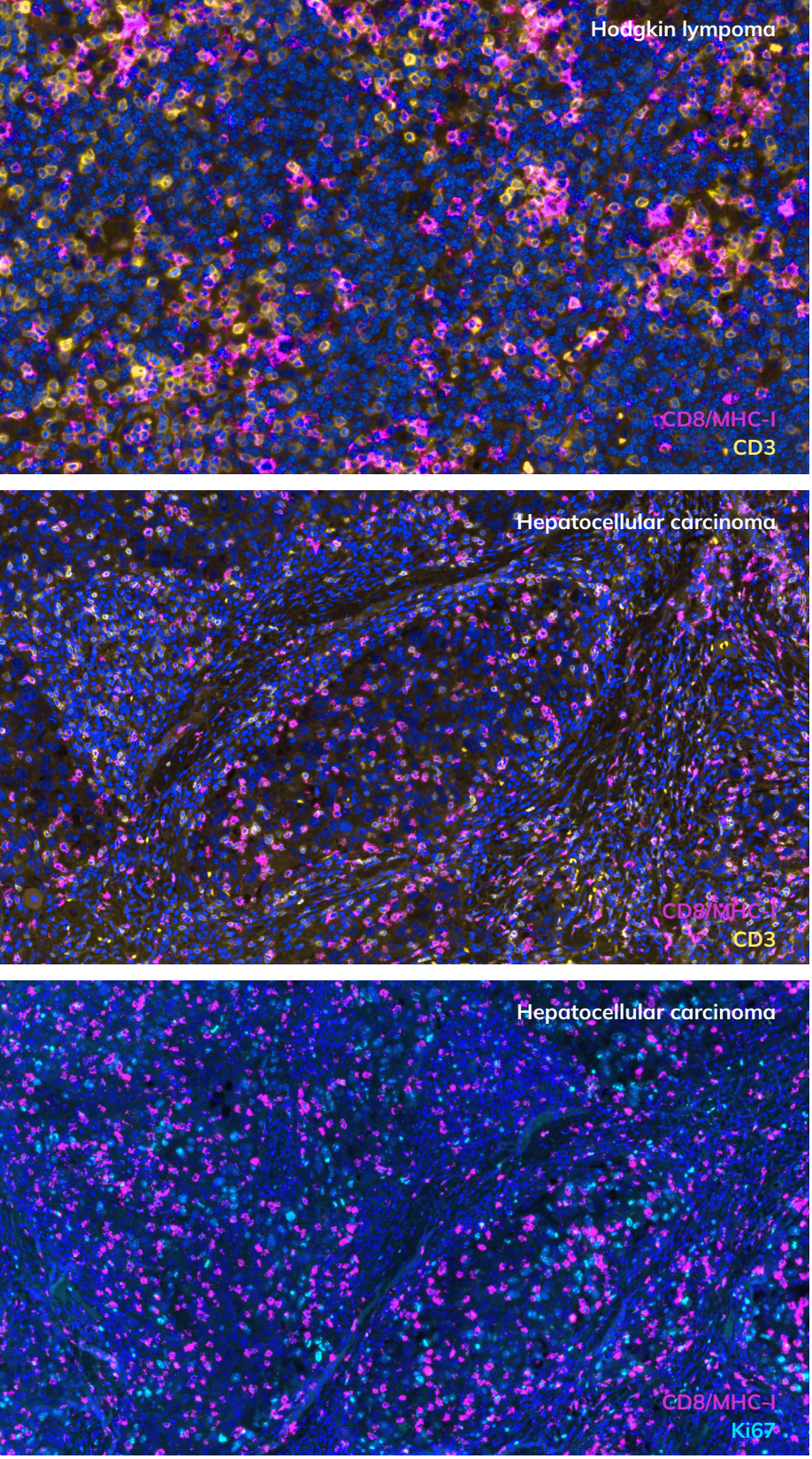


Fig. 3. CD8/MHC-I interaction staining with Naveni® CD8/MHC-I Atto647N and IF co-staining for CD3 or Ki67 in FFPE Hodgkin lymphoma and hepatocellular carcinoma. CD8/MHC-I (magenta) signal is present in the same regions as CD3 and Ki68 staining but is less abundant.

Conclusion

The CD8/MHC-I interaction is central to the delicate balance between immune protection against cancer and the prevention of autoimmune responses. Understanding the molecular and cellular mechanisms involved in this interaction in the contexts of healthy immune response, cancer and autoimmunity is essential for the development of targeted and effective immunotherapies that can either enhance antitumor immune responses or alleviate autoimmune pathologies. Ongoing research in this field holds promise for the discovery of novel therapeutic interventions with greater specificity and efficacy.

References

Burnet, F. M. 1970. “The Concept of Immunological Surveillance.” In *Progress in Tumor Research*, edited by R.S. Schwartz, 13:1–27. S. Karger AG. https://doi.org/10.1159/000386035.

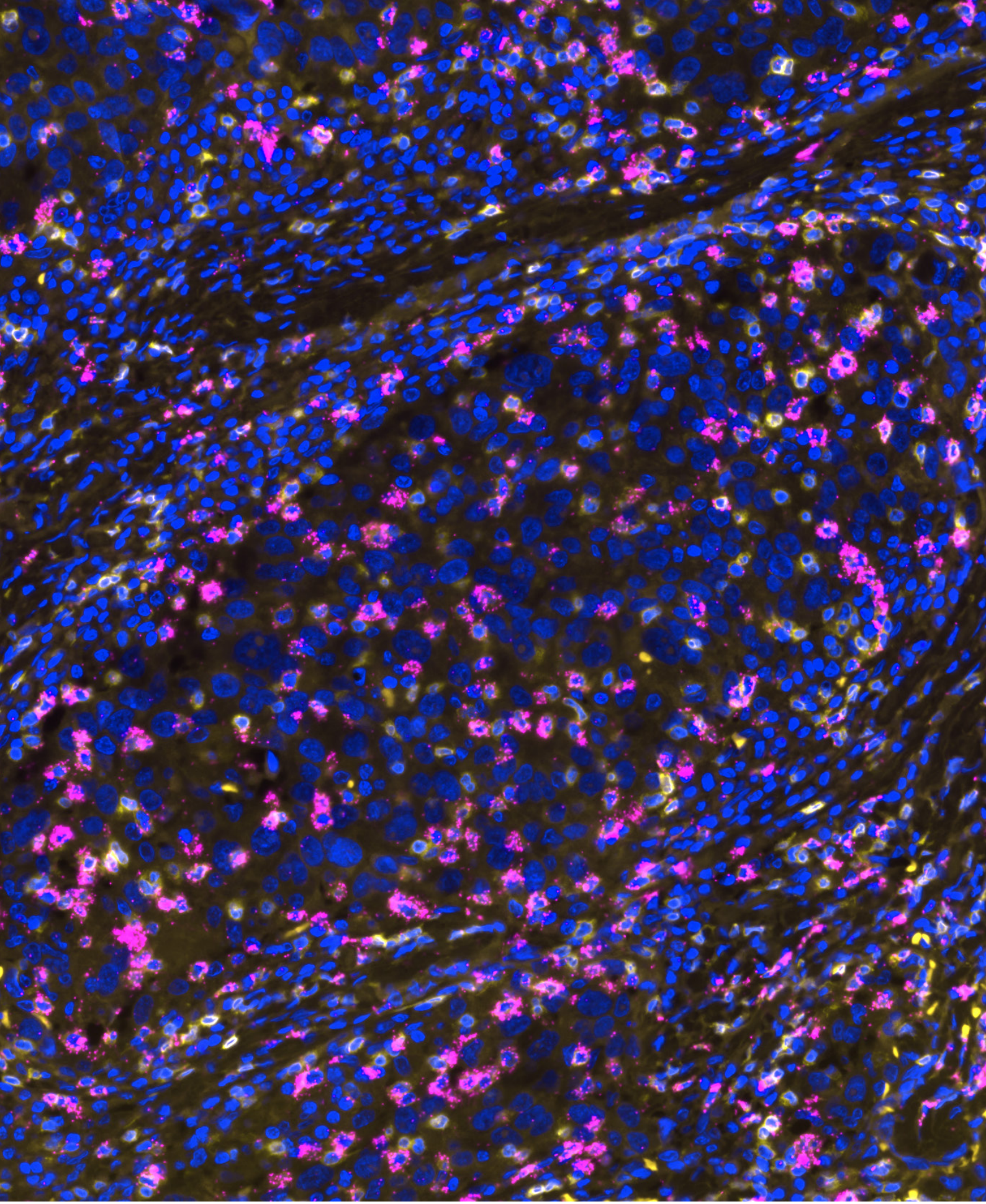
Chan, Jack D., Junyun Lai, Clare Y. Slaney, Axel Kallies, Paul A. Beavis, and Phillip K. Darcy. 2021. “Cellular Networks Controlling T Cell Persistence in Adoptive Cell Therapy.” *Nature Reviews Immunology* 21 (12): 769–84. https://doi.org/10.1038/s41577-021-00539-6.

Hewitt, Eric W. 2003. “The MHC Class I Antigen Presentation Pathway: Strategies for Viral Immune Evasion.” *Immunology* 110 (2): 163–69. https://doi.org/10.1046/j.1365-2567.2003.01738.x.

Pai, Joy A., Matthew D. Hellmann, Jennifer L. Sauter, Marissa Mattar, Hira Rizvi, Hyung Jun Woo, Nisargbhai Shah, et al. 2023. “Lineage Tracing Reveals Clonal Progenitors and Long-Term Persistence of Tumor-Specific T Cells during Immune Checkpoint Blockade.” *Cancer Cell* 41 (4): 776-790.e7. https://doi.org/10.1016/j.ccell.2023.03.009.

Walter, Ulrich, and Pere Santamaria. 2005. “CD8+ T Cells in Autoimmunity.” *Current Opinion in Immunology* 17 (6): 624–31. https://doi.org/10.1016/j.coi.2005.09.014.

Yang, Kailin, Ahmed Halima, and Timothy A. Chan. 2023. “Antigen Presentation in Cancer — Mechanisms and Clinical Implications for Immunotherapy.” *Nature Reviews Clinical Oncology* 20 (9): 604–23. https://doi.org/10.1038/s41571-023-00789-4.



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