Extended Abstracts for the 53rd International Symposium of the Princess Takamatsu Cancer Research Fund, 2025

CANCER IMMUNOLOGY IN EVOLUTION

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Princess Takamatsu Cancer Research Fund

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OPENING REMARKS

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The Princess Takamatsu Cancer Research Fund is the oldest and largest cancer research support organization in Japan, and is run as a purely private organization. The founder is Her Late Imperial Highness Princess Kikuko Takamatsu, who hailed from the Tokugawa Shogun's family.

At the age of 18, she married His Imperial Highness Prince Nobuhito Takamatsu, the third son of Emperor Taisho. In 1933, when she was 22, her mother, who was also a member of the Imperial Family, died of cancer at the age of 43. The Princess Takamatsu was keenly aware that there was still so much that was unknown about the disease of cancer, and she decided to devote her life to supporting cancer research.

In 1968, the Nadeshiko-kai (Flower Dianthus Group), a volunteer group made up of the Princess Takamatsu's alumnae from the Gakushuin Girls' School, established the Princess Takamatsu Cancer Research Fund to further strengthen support for cancer research. At the time, then-the Ministry of Education of the Japanese Government also actively supported the establishment of the first private foundation focusing on basic medical research. At the time of the foundation's establishment, the Princess Takamatsu was appointed as Honorary Patroness.

The main activities of the foundation include holding international symposia and lectureships, awarding academic prizes, and presenting research grants to Japanese researchers. Of these, the international symposia are the most important activity. At the time of the foundation's establishment, it was not easy for Japanese scientists to attend academic

conferences held overseas, but the foundation's international symposia provided young scientists with the opportunity to listen to lectures given by many internationally renowned cancer researchers, and contributed greatly to the development of cancer research in Japan. Later, the significance of holding international symposia, combined with the progress of cancer research in Japan, came to serve as a forum for exchange between Japanese cancer researchers and those from around the world.

The Princess Takamatsu passed away in 2004, and His Imperial Highness Prince Tomohito of Mikasa, who picked up the torch, made a precious contribution to the Fund's activities as Patron. The Prince Tomohito of Mikasa passed away in 2012, and since then, the foundation has continued to be operated under His Imperial Highness Prince Masahito Hitachi, who became Patron, and in 2018, the foundation celebrated the 50th anniversary. Looking back over the foundation's more than half a century history, the activities of this purely private organization have created a new trend in cancer research support, and the Japanese government has followed suit. Furthermore, it has become a model for all volunteer activities in Japan, and its role in pioneering medical research support has been highly evaluated.

The theme of the international symposium is decided by the Scientific Advisory Committee, chaired by Prof. Ryuzo Ueda. It was decided as "Cancer Immunology In Evolution" for the 53rd International Symposium. The preparations were carried out by the organizing committee, chaired by Dr. Hiroyoshi Nishikawa, with Dr. Sacha Gnjatic, Dr. Hiroyuki Mano, Dr. Naoko Ohtani and Dr. Yuki Kagoya serving as committee members.

Finally, I would like to introduce the words of the Princess Takamatsu, which expressed her thoughts on cancer research. I myself served as the attending physician to the Princess Takamatsu for about 20 years until her passing. During that time, Her Imperial Highness would confide to me on various occasions that "in our fight against cancer, the best results will ultimately be produced through the promotion of scientific research." I sincerely hope that the content presented at this symposium has given the attendees new ideas, that mutual exchange has inspired them for new researches, and that these activities will ultimately lead to the conquest of cancer.

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IMMUNOGENOMIC CANCER EVOLUTION: A FRAMEWORK OF IMMUNOSUPPRESSIVE MECHANISMS IN THE TUMOR MICROENVIRONMENT

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Aberrant signaling stemming from genetic abnormalities in cancer cells plays fundamental roles in carcinogenesis and escape from attack by the host immune system. Cancer cells must be immunologically selected and employ immunosuppressive mechanisms to become clinically apparent cancers. These mechanisms include decreased antigenicity; expression of various immunosuppressive molecules, such as immune checkpoint molecules; failure of maturation of antigen presenting cells; and accumulation of immunosuppressive cells [1, 2, 3]. Remarkable advances in new technologies that combine next-generation sequencing of DNA/RNA from human tumors and high-resolution immune phenotype profiling (Immuno-genomic analysis) have elucidated the relationships between the genetic abnormalities in cancers and the behaviors of immune cells in the tumor microenvironment (TME).

We have shown that regulatory T (Treg) cells with an activated phenotype, which are generally detected with effector T cells in the inflamed TME, highly infiltrated in *EGFR*-mutated lung adenocarcinomas although *EGFR*-mutated lung adenocarcinomas exhibited a non-inflamed TME. EGFR signals activated cJun/JNK and reduced IRF; the former increased CCL22 recruiting eTreg cells and the latter decreased CXCL10 and CCL5 inducing $CD8^+$ T-cell infiltration. EGFR signal inhibitors decreased Treg cell infiltration in the TME, and combination with immune checkpoint inhibitors (ICIs) provided better antitumor effects compared with either of single treatment [4].

While the TME is metabolically harsh for effector T cells, Treg cells can survive in this harsh environment, which rather enhances their immunosuppressive functions. FOXP3 suppresses MYC activity, which is a global regulator of glycolysis, leading to inhibition of

glycolysis and promotion of OXPHOS. Thus, the immunosuppressive function of Treg cells largely depends on mitochondrial respiration and OXPHOS. Accordingly, even in low-glucose and free fatty acid (FFA)-rich environments, the oxidative metabolism of lipids in Treg cells leads to resistance to FFA-induced cellular toxicity to support adaptation to the FFA-rich TME. Gastric cancer with *RHOA* Y42 mutations exhibited upregulated *FASN* expression and production of larger amounts of FFAs in the TME compared to tumors without *RHOA* Y42 mutations. This metabolic change caused by *RHOA* Y42 mutations in gastric cancer cells led to more abundant Treg cells with strong immunosuppressive functions and scarce effector T cells in the TME [5].

Moreover, Treg cells can utilize lactic acid (LA) in a high-LA environment. Treg cells metabolize LA to support their survival and immunosuppressive functions through MCT1, an LA transporter. We have previously shown that PD-1 expression by Treg cells is an important mechanism of resistance to PD-1/PD-L1 blockade therapy [6]. Therefore, we investigated cancer patient samples to address the correlation between PD-1 expression by Treg cells and metabolic pathways and found that *MYC*-amplified tumors and liver metastatic lesions established a LA-rich TME and promoted PD-1 expression by Treg cells in the TME via LA metabolism. When Treg cells took up LA from the microenvironment via MCT1, which is positively regulated by FOXP3, LA was metabolized into phosphoenolpyruvate (PEP). PEP increased the calcium ion (Ca^{2+}) concentration in the cytoplasm and induced NFAT1 translocation into the nucleus, resulting in higher PD-1 expression by Treg cells, while these effects were not observed in effector T cells, such as $CD8^+$ T cells. In highly glycolytic tumors, including *MYC*-amplified tumors and liver metastatic lesions, combination treatment with ICI and molecular-targeted therapy related to lactic acid metabolism could overcome resistance to ICI treatment [7].

In certain patients with cancer, self-antigens abundantly present on MHC-II of cancer cells directly activated Treg cells and strongly suppressed antitumor immune responses. Copy number gain encoding self-molecules in cancer cells caused abundant immunogenic self-peptides to be presented to Treg cells, resulting in Treg cell activation in the TME, which was associated with a poor prognosis and resistance to ICI treatment.

Therefore, Treg cells are considered key targets for cancer immunotherapy. However, since they are also critical cells for maintaining self-tolerance, elucidating the characteristics of Treg cells in the TME and selectively targeting them is essential for the success of cancer immunotherapy. To this end, peripheral blood, lung normal tissues, and lung cancer tissues were collected from non-small cell lung cancer patients. Comprehensive molecular expression analysis was performed at the single-cell level to investigate the molecular profile of Treg cell differentiation and activation. Treg cells were classified into 11 distinct fractions, and Treg cells in the TME possessed a distinct chromatin profile and gene expression pattern

compared to other effector T cell subsets and peripheral blood Treg cells. More importantly, BATF was identified as a key regulator that controls Treg cell differentiation by epigenetically regulating the expression of activation-associated genes, thereby conferring robustness to Treg cells in the TME. Using a mouse model with selective knockout of the BATF gene in Treg cells, we demonstrated that the number and suppressive activity of Treg cells in the TME were significantly reduced, and their characteristic chromatin structure and gene expression was lost. Thus, BATF deficiency in Treg cells remarkably inhibited tumor growth, and high BATF expression was associated with poor prognosis in patients with lung cancer, kidney cancer, and melanoma. One of the specific chromatin remodeling and differentiation programs of Treg cells in the TME can be applied to the development of Treg cell–targeted therapies [8].

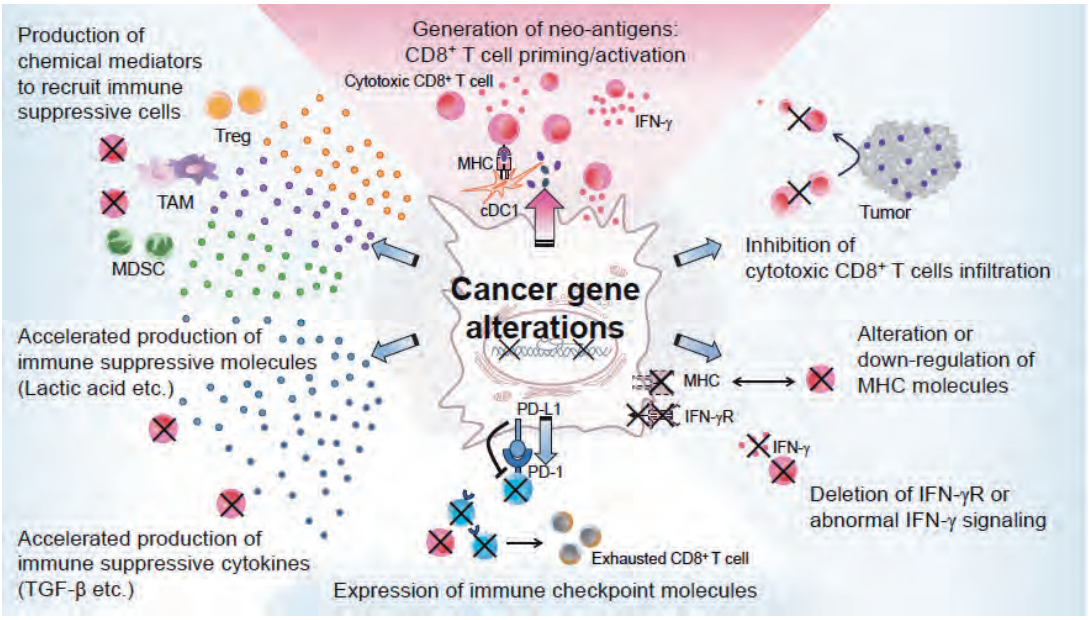

Figure 1 Driver gene alterations, which can become neoantigens, directly modulate the chemokine milieu and recruit immunosuppressive cells. These alterations provide metabolic support promoting the survival and activation of immunosuppressive cells in the TME. and directly activate immunosuppressive cells by expressing MHC class II by cancer cells in an aberrant manner.

Altogether, during cancer development and growth, which is known as the process of cancer initiation, promotion, progression and metastasis (the evolutionary theory of cancer), the immune system also plays a critical role in allowing its diversification and selection; oncogenic aberrant signaling is involved in both carcinogenesis and immune evasion (Figure

1) (Immunogenomic cancer evolution). Thus, inhibition of oncogenic signaling by specific driver oncogenes has been shown not only to kill cancer cells but also to augment antitumor immunity, suggesting the potential for the advent of molecularly targeted reagents with multiple immunomodulatory functions from the perspective of personalized therapies (Immunogenomic precision medicine).

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CHEMOKINES THAT ORGANIZE CELLULAR COMMUNICATION IN THE TUMOR MICROENVIRONMENT

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Effective immune responses, including those directed at malignant tumors, depend on the intricately orchestrated interactions of our various types of immune cells to coordinate their proliferative expansion, differentiation, and ultimately, execution of different immunological effector activities against cancer cells. These interactions generally occur across short distances via soluble factors (e.g., cytokines) or through direct cell-cell contact, both of which require proximal positioning of interacting cells. Immune cell positioning, in turn, is organized through chemotactic factors, most prominently chemokines, produced and released by both immune cells as well as non-hematopoietic cells to form tissue microenvironments and niches that organize cellular neighborhoods at a range of spatial scales. While we now have some understanding of how these cellular interactions are orchestrated in lymphoid tissues during the initiation of immune responses, we have only rudimentary knowledge of how immune cells are positioned and immune responses sustained and amplified in tumor microenvironments (TME) [1]. Such knowledge is however critical to understand reasons for failure of existing treatments and to design more effective cancer immuno-therapies.

In prior studies, we have discovered that the local proliferative expansion and survival of cytotoxic $CD8^+$ effector T cells ($CD8^+$ TEF) in the TME depends on their interactions with a $Ccr7^+$ dendritic cell (DC) state that densely clusters around blood vessels of the tumor stroma and *trans*-presents the T cell survival cytokine IL-15 [2]. These interactions depend on T cell-expression of the chemokine receptor Cxcr6, which binds its ligand Cxcl16 most highly expressed by perivascular $Ccr7^+$ DCs. $CD8^+$ T cells that lack Cxcr6 are unable to locally expand in the TME and fail to control tumor growth [2] (**Figure 1**). These observations have

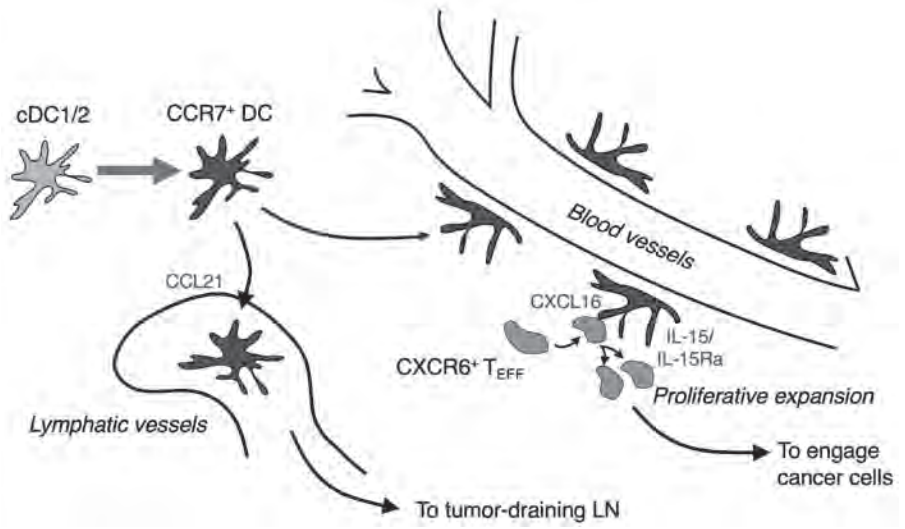

Figure 1 Some Ccr7$^+$ DCs of the TME are diverted to perivascular stromal niches, where they attract Cxcr6$^+$ effector T cells and *trans*-present them with IL-15 to support their local proliferative expansion before engaging with cancer cells

led us to explore the TME more widely for chemokines and their receptors and their role in facilitating functional cross-talk between immune cells, stroma cells, and cancer cells.

In recent preliminary studies we have used single cell spatial transcriptomics in mouse models of cancer to discover that perivascular DC clusters not only express Cxcl16, but are also the most abundant source in the tumor stroma of other T cell-attracting chemokines, including Ccl5 and Ccl22, and are at the core of a wider assembly not only of CD8$^+$ TEF, but also of Tcf-1$^+$ CD8$^+$ stemlike T cells, conventional, and regulatory CD4$^+$ T cells. Using a novel genetic mouse model that enables us to specifically ablate Ccr7$^+$ DCs, we found that these cells retain CD4$^+$ and CD8$^+$ T cells in the tumor stroma of mouse melanoma tumors and that in their absence, T cells readily invade the tumor core. Therefore, tumor immune exclusion does not only result from physical or functional barriers that hinder immune cell entry but can also be caused by their retention by chemokine-secreting perivascular dendritic cells in the tumor stroma.

The canonical role of the chemokine receptor Ccr7 on tumor-associated dendritic cells is to enable them to enter lymphatic vessels and traffic to draining lymph nodes where they prime tumor antigen-specific T cells (**Figure 1**). This raised the question of what mechanisms divert some Ccr7$^+$ DCs away from lymph vessels and towards perivascular niches of the tumor stroma. Using spatial transcriptomics to characterize chemokine expression of cancer-associated fibroblasts (CAFs), we found that a subsets of CAFs expressing the cell contractility regulator Tagln align closely with blood vessels and express the Ccr7 ligand

Ccl19, suggesting that CAF-derived Ccl19 attracts or retains $Ccr7^+$ DCs in perivascular niches. Indeed, genetic deletion of Ccr7 prevented DCs from clustering around stromal microvessels. Thus, local effector T cell expansion in solid tumors requires CAF-secreted Ccl19 to guide $Ccr7^+$ DCs to perivascular niches, and $Ccr7^+$ DCs in turn use Cxcl16 to attract $CD8^+$ T_{EFF} but also express other T cell-attracting chemokines and retain T cells in the peritumoral stroma.

In this presentation, I will examine the TME more widely for the roles of chemokines in locally orchestrating anti-tumor immunity.

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2000-2002	Post-doctoral Fellowship, Institute for Surgical Research, Ludwig-Maximilians University
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PANCREATIC CANCER – SURVIVORS TO SOLUTIONS

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In 2017, we found that rare long-term survivors of pancreatic cancer naturally mount $CD8^+$ T cell-driven anti-tumor immunity to neoantigens (Balachandran et al., 2017). This discovery challenged the prevailing assumption that pancreatic cancer, like most solid tumors, is immunologically cold and lacks clinically relevant neoantigens, and sparked efforts to "therapeutically phenocopy" the exceptional survivor state. Here, I will outline our progress to translate immunological insights from exceptional survivors to the clinic. Specifically, I will emphasize recent findings on two complementary approaches to activate immunity in pancreatic cancer: (1) enhance tumor antigen recognition and (2) boost de novo lymphoneogenesis in the tumor microenvironment.

Enhance tumor antigen recognition: We discovered long-term pancreatic cancer survivors have tumors enriched in T cells that recognize "high quality" clinically relevant neoantigens (Balachandran et al., 2017; Luksza et al., 2017; Luksza et al., 2022). As the most successful way to boost immune response to an antigen is with a vaccine, we designed and executed a landmark clinical trial of bespoke RNA neoantigen vaccines in patients with resectable pancreatic cancer. Our results show these vaccines are safe and immunogenic (Rojas et al., 2023), with deep longitudinal analysis revealing vaccines can induce $CD8^+$ T cells of substantial magnitude, longevity, and function, which correlate with delayed recurrence at 3- year follow-up (Sethna et al. *Nature*, 2025). As long-lived functional vaccine-induced $CD8^+$ T cells are essential for effective cancer vaccines, our results suggest such bespoke neoantigen vaccination approaches could provide blueprints with which to harness $CD8^+$ T cells against cancer and other diseases.

To build on these proof-of-principle studies in pancreatic cancer and extend concepts to

other high unmet-need cancers, in 2024, we established a cancer vaccine translational research hub at MSK – The Olayan Center for Cancer Vaccines (OCCV; mskcc.org/occv).

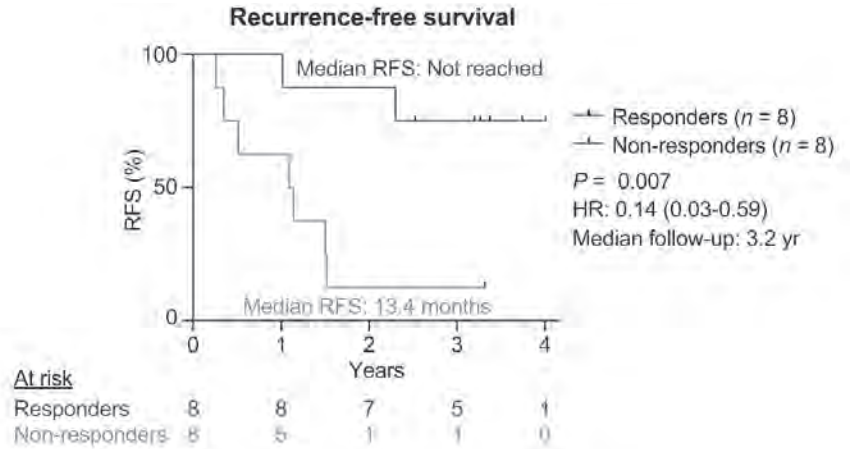

Figure 1 Recurrence-free survival (RFS) stratified by vaccine response. Sethna et al. *Nature*, 2025.

Boost de novo lymphoneogenesis: In complementary work to activate immunity in pancreatic cancer, we discovered a new potentially druggable pathway of de novo lymphoneogenesis. Specifically, we found that the cytokine IL-33 can activate a unique type of immune cell enriched in long-term survivors, group 2 innate lymphoid cells (ILC2s) (Moral et al., 2020), and $CD8^+$ T cells to boost anti-tumor immunity. Further mechanistic studies in mice revealed this is mediated through formation of tertiary lymphoid structures (TLSs) (Amisaki et al. *Nature*, 2025; Figure 2), revealing that IL33-activated ILC2s express the lymphoneogenic molecule lymphotoxin, and engage lymphotoxin receptor expressing myeloid cells to induce ectopic lymphoid aggregates that regulate immunity in chronically inflamed tissues, including tumors (**Figure 2**). Interestingly, we observed these lymphoneogenic ILC2s are not tissue-resident like most ILC2s but are in fact migratory, mobilizing to sites of inflammation including tumors with IL33 from ILC2 reservoirs such as the gut (**Figure 2**). To translate our

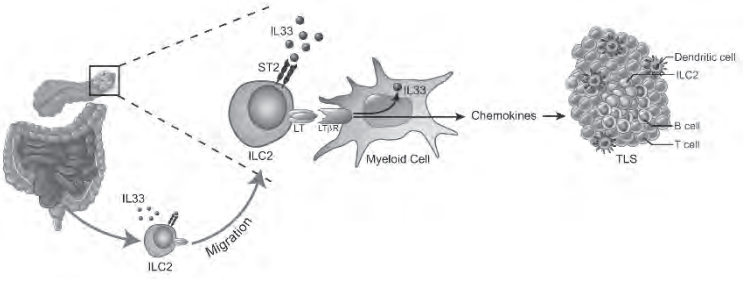

Figure 2 The IL33-ILC2-TLS pathway. Amisaki et al. *Nature*, 2025

findings, we have now designed an optimized human IL33 fusion protein drug candidate for imminent first-in-human clinical testing.

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Neoantigens:

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2017-2020	Laboratory Head, Memorial Sloan Kettering Hospital Research Laboratories
2020-2023	Assistant Member and Laboratory Head, Immuno-Oncology Service, Human Oncology and Pathogenesis Program, MSK
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2023-present	Associate Professor of Surgery, Weill Cornell Medical College
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2025-present	Hutham S. Olayan and Robert F. Raucci Chair, MSK

NOT ALL ANTI-TUMOR IMMUNITY IS EQUAL: HOW DENDRITIC CELLS SHAPE T CELL RESPONSES

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The clinical success of immune checkpoint blockade (ICB) has established the principle that the immune system, particularly cytotoxic CD8+ T cells, can be harnessed to recognize and eradicate disseminated tumor cells. However, most patients fail to respond to ICB, largely due to insufficient infiltration of functional cytotoxic T cells into the tumor microenvironment. While the presence of CD8+ T cells is necessary, the functional quality of these cells—defined by their differentiation and effector potential—is increasingly recognized as critical to a productive anti-tumor response. Canonical T cell exhaustion represents one well-characterized dysfunctional state that remains responsive to ICB [1]. Yet, not all T cell dysfunction can be attributed to exhaustion, and emerging evidence highlights the existence of distinct, ICB-refractory dysfunction programs that are poorly understood [1].

Dendritic cells (DCs), as key antigen-presenting cells, critically shape T cell differentiation during both priming in lymphoid tissues and restimulation in tumors. However, the molecular and environmental determinants that govern DC:T cell interactions across different tissue sites remain incompletely defined. My research program aims to elucidate how tissue- and tumor-specific factors modulate the quality of DC-mediated T cell responses, with the goal of uncovering mechanisms underlying immune dysfunction and identifying opportunities to restore anti-tumor immunity in ICB-resistant cancers.

To this end, we have undertaken a comprehensive investigation of tissue-specific anti-tumor immunity using both clinical observations and mechanistic mouse models. Tumors arising in different tissues, such as the lung, skin, ovary, or brain, display distinct immune responsiveness to ICB, suggesting that tissue microenvironments influence the trajectory of anti-tumor immunity. Our work in non-small cell lung cancer (NSCLC) has identified a

unique dysfunctional T cell state—termed TLdys (tolerance-like dysfunction)—which is associated with T cell priming in the lung-draining mediastinal lymph node [2-4]. These T cells exhibit activation and expansion yet fail to differentiate into effector or exhausted states. Notably, this dysfunction is established within 72 hours of priming and is driven by regulatory T cells (Tregs) and elevated interferon-gamma levels specific to the lung-associated lymph node [2]. While unresponsive to ICB, TLdys T cells can be functionally restored by combined IL-2 and IL-12 cytokine therapy, revealing a therapeutic opportunity for ICB-refractory tumors [3].

Extending our findings to other poorly responsive cancers, we have uncovered similarly tissue-restricted immune suppression mechanisms in ovarian cancer and glioblastoma. In ovarian cancer, we identified a novel tolerogenic DC population enriched in peritoneal lesions that directly suppresses T cell responses. In glioblastoma, we are currently investigation how priming affects anti-tumor immunity. These studies collectively underscore the importance of the priming microenvironment in determining T cell fate and provide a framework for understanding immune evasion across diverse tumor types.

In parallel, we are investigating how tumor cell-intrinsic properties influence the local immune landscape. Tumor-driven exclusion of T cells and DCs, loss of antigenicity, and immune editing are established modes of immune escape. However, our studies have revealed novel tumor-intrinsic programs that promote or suppress immunity via their impact on DC phenotype and function. For example, we have shown that spontaneous tumor control can occur independently of canonical cross-presenting DC1 cells [5]. Instead, an alternative DC2 activation state—marked by high co-stimulatory molecule expression and IFN-β-dependent cross-dressing of tumor-derived MHC-I—can initiate effective T cell responses. These ISG+ DC2 cells contribute to durable cytotoxic T cell immunity and represent a non-canonical pathway of DC-mediated activation that may be therapeutically leveraged [5].

We have also demonstrated that specific oncogenic drivers, such as Sox2, facilitate T cell exclusion by promoting Treg recruitment and impairing vascular maturation, thus preventing T cell infiltration [6]. Furthermore, our studies on neoantigen heterogeneity reveal that the clonal versus subclonal distribution of antigens affects their presentation by DC subsets and the resultant T cell responses [7,8]. Clonal neoantigens presented by mature DC1 cells elicit strong T cell activation, whereas subclonal antigens are presented by less mature DCs, leading to suboptimal responses. Importantly, targeted vaccination against subdominant neoantigens can overcome this hierarchy and synergize with ICB, offering a strategy to bypass antigen-dominance constraints [7].

Overall, our research establishes that both tissue context and tumor cell-intrinsic features play pivotal roles in shaping the functionality of anti-tumor T cell responses through their

influence on DC:T cell interactions. These findings redefine our understanding of immune responsiveness and resistance in cancer and open new avenues for precision immunotherapy. Ongoing efforts focus on elucidating how conventional treatments such as chemotherapy and surgery intersect with these immunological pathways, and on optimizing therapeutic vaccine design to exploit our growing knowledge of DC biology and T cell priming dynamics.

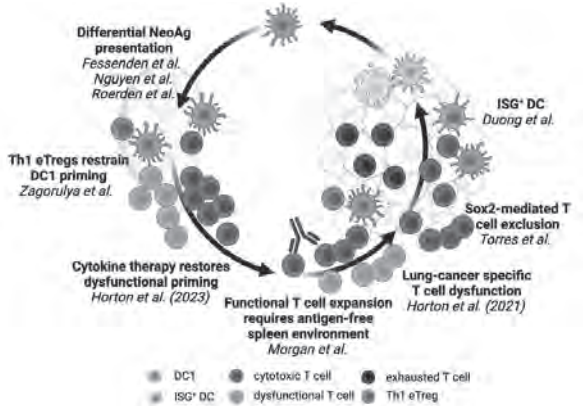

Figure 1 Overview of the publications by the Spranger group with the goal to understand anti-tumor immunity in the context of different microenvironments.

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NEOADJUVANT IMMUNOTHERAPY WITH TLR9 AGONIST AND ANTI-PD-1 IN MELANOMA

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Background: Vidutolimod is a novel virus-like particle (VLP) composed of type A CpG G10 encapsulated within a highly immunogenic Qβ bacteriophage capsid. Preclinical data suggest that anti-Qβ antibodies promote uptake by plasmacytoid dendritic cells (pDCs) and monocytes, mediating potent anti-tumor responses. In immune checkpoint inhibitor-refractory melanoma, intratumoral vidutolimod demonstrates single-agent activity, and its combination with pembrolizumab has shown durable responses and manageable toxicity.
Hypothesis: We hypothesized that combining intratumoral vidutolimod with systemic PD-1 blockade would synergistically convert immunologically "cold" tumors into "hot" ones by recruiting and activating tumor-infiltrating pDCs, thus enhancing T cell–mediated immunity and improving outcomes over anti-PD-1 monotherapy.
Study Design: A phase II neoadjuvant trial was conducted in clinical stage III melanoma, evaluating intratumoral vidutolimod in combination with systemic nivolumab.
Key Clinical Findings:

- **Pathologic Response:** Neoadjuvant vidutolimod /nivolumab achieved a 45% pathologic complete response (pCR) and a 55% major pathologic response (MPR)—exceeding rates observed in neoadjuvant anti-PD-1 monotherapy and comparable to anti-PD-1/CTLA-4 and nivolumab/relatlimab combinations.
- **Toxicity:** The regimen was well-tolerated with no dose-limiting toxicities or grade 4/5 treatment-related adverse events. Grade 3 TRAEs occurred in 7 patients, mostly manageable hypertension.
- **Survival:** MPR was associated with impressive 2-year outcomes: RFS of 88% and DMFS of 94%—exceeding those from adjuvant anti-PD-1 or talimogene laherparepvec

therapies.

Mechanistic Insights:

1. **Tumor Microenvironment Remodeling:** MPR tumors showed enriched $CD8^+$ TILs, TLS formation, necrosis, and melanophagocytosis, with transcriptional evidence of increased myeloid, pDC, and macrophage activation signatures—suggesting unique vidutolimod-driven modulation of the immune microenvironment.
2. **pDC Infiltration and Recruitment:** Spatial transcriptomics and GeoMx DSP confirmed robust peri-tumoral pDC accumulation in MPRs. Recruitment was potentially mediated by CXCR3 ligands and CXCL12 in response to intratumoral CpG, supporting the role of pDCs in anti-tumor immunity.
3. **Peripheral Immune Activation:** Peripheral blood from MPR patients exhibited early increases in $Ki67^+CD8^+$ and $CD4^+$ T cells, as well as activated non-classical monocytes and pDCs, characterized by reduced PD-1/PD-L1 expression—highlighting systemic immune activation beyond what is typically observed with PD-1 blockade alone.
4. **Microbiome Composition:** Gut microbiome profiles associated with vidutolimod/nivolumab response diverged from those linked to favorable anti-PD-1 monotherapy outcomes, suggesting treatment-specific microbial correlates of efficacy. The clinical findings align with those from murine models, where intratumoral type B CpG-ODN induces necrosis, recruitment of $Ly6C^+$ $MHC\text{-}II^+$ DCs, and robust adaptive immune responses, even in the absence of an optimal microbiome.

   Conclusions: Neoadjuvant intratumoral vidutolimod plus systemic nivolumab is a promising strategy in resectable stage III melanoma, yielding high rates of pathologic response, excellent early survival outcomes, and a favorable safety profile. Unique innate and adaptive immune mechanisms—including pDC recruitment, myeloid reprogramming, and peripheral T cell activation—distinguish vidutolimod / nivolumab from conventional PD-1 blockade and support its further development.

   Future Directions:

   Single-cell RNA sequencing and spatial transcriptomics are underway to further elucidate the tumor and peripheral immune dynamics associated with MPRs. These studies aim to refine the mechanistic understanding of vidutolimod's additive benefit and identify predictive biomarkers of response.

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Personal Statement

I am a Dermatologist and a Cancer Immunologist with a long-lasting interest in immunotherapies of melanoma, cancer vaccines, and human T cell immunology. I am co-leader of the Cancer Immunology and Immunotherapy Program (CIIP). I lead the translational research efforts within CIIP, with a focus on promoting the translation of basic scientific discoveries into early-phase clinical trials, accompanied by correlative immunological studies. I co-lead the Melanoma and Skin Cancer SPORE of the University of Pittsburgh. My research program focuses on novel immunotherapies of melanoma, T cell responses to melanoma antigens, and the mechanisms of melanoma-induced T cell dysfunction. I have made seminal contributions in the field of human cancer immunology, identifying multiple novel tumor antigen-specific CD4 epitopes, determining the role of the novel inhibitory receptor pathways Tim-3 and TIGIT in impeding T cell responses to melanoma, and dissecting the mechanisms of action of neoadjuvant CPG intratumoral together with PD-1 blockade in melanoma. These findings have been translated into multiple first-in-human clinical trials in melanoma and other solid tumors, including cancer vaccines composed of CD4 epitopes and adjuvants (CPG), neoadjuvant intratumoral CPG and PD-1 blockade, and third-generation immune checkpoint blockades with anti-TIM-3 or anti-TIGIT antibodies, combined with anti-PD-1 antibodies. I have led translational studies evaluating the role of the gut microbiome in regulating clinical responses to PD-1 blockade in melanoma, including a first-in-human clinical trial to assess the safety and efficacy of fecal microbiota transplant (FMT) obtained from long-term PD-1 responder patients and pembrolizumab in patients with PD-1 refractory melanoma. We have identified intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. We have also demonstrated that specific gut microbial communities (enterotypes) with non-uniform geographical distributions are associated with either favorable or unfavorable clinical outcomes.

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TARGETING STAT3 IN DENDRITIC CELLS TO BOOST TUMOR IMMUNITY AND IMMUNOTHERAPY

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Immunotherapy, including checkpoint blockade (ICB) and T cell therapy, utilizes the immune system to target and eliminate cancer cells (1-3). However, most patients are either poorly responsive to ICB or develop therapeutic resistance. Emerging evidence suggests that ICB fails to trigger potent immune responses in patients with limited and impaired dendritic cells (DCs), particularly DC1s (4), in the tumor microenvironment (TME). DCs are professional antigen presenting cells (APCs), presenting tumor antigens to prime and activate T cells, thereby driving anti-tumor immune responses (5-10). Increased DC1 infiltration correlates with prolonged survival in patients with cancers (11-13). However, the TME can impede DC1 maturation and function, partly due to disruptive environmental signals (7, 14-16). Thus, understanding how the DC function is shaped in the TME and exploring strategies to enhance DC1 function are crucial for developing more effective cancer immunotherapy.

STAT pathways control immune cell phenotype and function in the TME (17). STAT3 is frequently activated in many cancer types by the JAK kinases, inhibiting immune response in the TME (18, 19). STAT3 activation leads to the production of various protumor factors, including VEGF and IL-6, impeding DC maturation and function (20). Moreover, STAT3 signaling can inhibit Th1-type chemokine expression and subdue DC tumor trafficking, resulting in the exclusion of T cells from the TME (21). In contrast, STAT5 is activated in response to cytokine signals, such as GM-CSF and IL-2 (22), and plays a positive role in the anti-tumor immune response (23, 24). On this basis, we hypothesized that the balance and dynamics between the STAT5- and STAT3-transcriptional pathways in DCs may shape the fate of distinct immune responses in the TME, in turn determining ICB responses in patients

with cancer. To test this hypothesis, we analyzed single cell-sequencing and bulk RNA-sequencing datasets from tumor tissues in cancer patients receiving ICB. We discover ICB reprogrammed the interplay between the STAT3- and STAT5-pathways in DCs, thereby activating T-cell immunity and enabling ICB efficacy. Mechanistically, STAT3 restrained the JAK2- and STAT5- pathway, determining the fate of DC function. We designed two types of specific PROTAC degraders of STAT3, SD-36 and SD-2301. STAT3-degraders effectively degraded STAT3 in DCs and reprogramed the DC-transcriptional network toward immunogenicity. Furthermore, STAT3-degrader monotherapy was efficacious in treating advanced tumors and ICB-resistant tumors without toxicity in mice. Thus, the crosstalk between the STAT3- and STAT5-transcriptional pathways determines the DC phenotype in the TME and determines the outcome of cancer immunotherapy. Our study provides proof-of-concept that pharmacological degradation of STAT3 can treat multiple tumor types as monotherapy and sensitize tumors to ICB (25).

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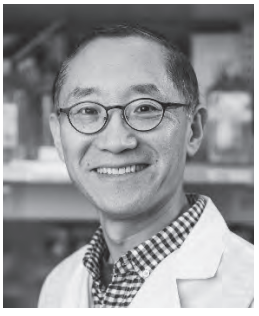

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ELUCIDATING REGULATORY MECHANISMS OF T CELL ACTIVATION BY IMMUNE CHECKPOINT MOLECULES

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T cell activation initiated by antigen-dependent signal through the T cell receptor is finely regulated by antigen-independent inputs from stimulatory and inhibitory co-receptors to promote effective immune responses while preventing autoimmunity and other harmful consequences. Therapeutic blockade of inhibitory co-receptors–now widely recognized as immune checkpoint molecules, such as PD-1 and CTLA-4–has revolutionized cancer treatment, producing durable clinical responses in multiple malignancies. However, response rates remain limited, and a considerable proportion of patients experience immune-related adverse events, underscoring the need for novel therapeutics with improved efficacy and safety. This has prompted increasing interest in targeting alternative inhibitory co-receptors.

We have been focusing on LAG-3, as we previously demonstrated its functional cooperation with PD-1 in regulating T cell responses [1]. Although LAG-3 is currently recognized as a potent inhibitory co-receptor, many of its fundamental properties remain incompletely understood. Earlier studies suggested that LAG-3 inhibits the activation of CD4 T cells by outcompeting CD4 for binding to MHC class II (MHCII). However, we found that LAG-3 does not recognize MHCII universally and that its inhibitory function is independent of CD4. Our subsequent work revealed that LAG-3 selectively binds structurally stable peptide–MHCII complexes (pMHCII), which are generated with the assistance of invariant chain and H2-DM [2]. Through this mechanism, LAG-3 suppresses CD4 T cells recognizing stable pMHCII, thereby regulating autoimmunity and anti-tumor immunity [3].

Likewise, PD-1 engages in closer functional cooperation with other co-receptors than

previously appreciated. While the inhibitory effect of PD-1 is more prominent during the effector phase than during the activation phase of T cell responses, the regulatory mechanisms underlying this phase-specific activity have been unknown. We demonstrated that PD-1 function is restricted during the activation phase by cis-PD-L1–CD80 interactions that disrupt PD-L1–PD-1 binding [4]. CD80 thus plays a dual role–activating CD28 co-stimulatory signal while simultaneously restricting PD-1 co-inhibitory signals. Notably, continuous activation of T cells by antigen presenting cells expressing high levels of CD80 and PD-L1 is required for both autoimmune disease development and tumor eradication, and the disruption of this restriction alleviates autoimmune symptoms in multiple murine models [5].

Beyond this T cell-extrinsic regulation of PD-1 via ligand availability, we identified T-cell-intrinsic factors that shape PD-1 function. Specifically, PD-1 preferentially suppresses the expression of genes with higher activation thresholds (i.e., genes with higher EC50 of stimuli for up-regulation) during T cell stimulation [6]. Accordingly, T cells with lower affinity for antigen were found to be more susceptible to PD-1-mediated inhibition [7]. Thus, PD-1 activity is jointly regulated by T-cell-intrinsic and -extrinsic mechanisms that alter T cell responsiveness and PD-1 ligand availability [8].

In this presentation, I will discuss recent advances in elucidating the molecular mechanisms of immune checkpoint function, which may provide new insights into immune modulation and guide the development of innovative strategies to improve clinical outcomes in cancer and other immune-related diseases.

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MECHANISTIC INSIGHTS INTO GUT MICROBIOTA-MEDIATED ENHANCEMENT OF PD-1 BLOCKADE THERAPY

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Immune checkpoint inhibitors (ICIs) have become a standard treatment for many cancers, and ICI-based combination therapies are now widely implemented. However, a substantial proportion of patients still fail to respond, with durable clinical benefit observed in only approximately 20% of cases. One of the key determinants of ICI efficacy is the extent of intratumoral infiltration by activated $CD8^+$ T cells, particularly $PD\text{-}1^+$ $CD8^+$ T cells. This infiltration is largely shaped by the tumor immune microenvironment (TIME), with inflamed tumors exhibiting higher T cell infiltration and improved therapeutic outcomes.

The gut microbiota has also emerged as a critical modulator of ICI response. While diversity and specific bacterial taxa have been linked to treatment outcomes, how gut microbes influence the TIME or $CD8^+$ T cell infiltration in distant tumors remains unclear.

In this presentation, I will outline the principal discoveries identified in our research.

1) Gut microbial features associated with ICI efficacy: Enrichment of *Ruminococcaceae* in responders

We analyzed 50 patients with non-small cell lung cancer (NSCLC) or gastric cancer (GC) to explore the link between gut microbiota composition and anti–PD-1 efficacy. Using pretreatment tumor and fecal samples, we performed flow cytometry of tumor-infiltrating immune cells and conducted 16S rRNA sequencing of the gut microbiota. Responders (Rs) showed a significant enrichment of *Ruminococcaceae*, while non-responders had more *Bacteroidaceae*. Higher *Ruminococcaceae* abundance was associated with longer progression-

free survival (PFS), whereas *Bacteroidaceae* dominance correlated with shorter PFS. Importantly, Ruminococcaceae-rich patients had greater intratumoral infiltration of PD-1$^+$ CD8$^+$ T cells, indicating a strong association between gut microbial composition, antitumor immunity, and ICI efficacy.

2) Identification of a novel gut bacterium "YB328" that enhances ICI efficacy

To identify gut bacteria that modulate anti–PD-1 response, we isolated *Ruminococcaceae* strains from fecal samples of Rs and successfully cultured a previously unreported strain, designated "YB328". As a control, *Phocaeicola vulgatus* was isolated from NRs. In antibiotic-treated SPF mice, YB328 combined with anti–PD-1 antibody induced significant tumor regression, increased PD-1$^+$ CD8$^+$ T cells in the gut, and upregulated dendritic cell (DC) activation markers. Importantly, co-administration of YB328 with NR-derived feces restored anti–PD-1 efficacy. T cell receptor (TCR) repertoire analysis showed that YB328 broadened TCR diversity.

3) YB328 promotes dendritic cell activation and enhances T cell responses

Given the evidence that YB328 activates DCs, we conducted mechanistic studies using bone marrow–derived dendritic cells (BMDCs). YB328 stimulation significantly increased expression of the maturation markers compared to *P. vulgatus*. In co-culture experiments using OVA-pulsed BMDCs and CD8$^+$ OT-I T cells, BMDCs stimulated with YB328 exhibited enhanced dendritic arborization and prolonged interaction with antigen-specific T cells. Using high- (N4) and low-affinity (Q4H7) OVA peptides, we found that even low-dose N4 peptides triggered nuclear translocation of the transcription factor NFATc1 and induced PD-1$^+$ CD8$^+$ T cells under YB328 stimulation—an effect not seen with *P. vulgatus*. These results demonstrate that YB328 robustly activates innate DCs and enhances both the quality and magnitude of CD8$^+$ T cell responses.

4) YB328 induces CD103$^+$ dendritic cells via TLR signaling and drives antitumor immunity

To elucidate why YB328-stimulated BMDCs elicit stronger CD8$^+$ T cell responses than *P. vulgatus*, we performed RNA-seq profiling. YB328 stimulation led to strong upregulation of *Batf3* and *Irf8*, transcription factors required for differentiation of conventional type 1 dendritic cells (cDC1), which express CD103 and specialize in cross-presentation. In *Batf3*-deficient mice lacking CD103$^+$ DCs, YB328 failed to exert any antitumor effect, underscoring the essential role of cDC1. Gene expression analysis revealed increased expression of multiple Toll-like receptors (TLRs). Accordingly, *MyD88*-knockout mice (lacking key TLR signaling) and *TLR7/9* double-knockout mice both failed to induce CD103$^+$ DCs or respond to YB328 therapy. Moreover, TLR agonists mimicked the antitumor effects of YB328,

highlighting the centrality of TLR signaling in mediating cDC1-driven antitumor immunity.

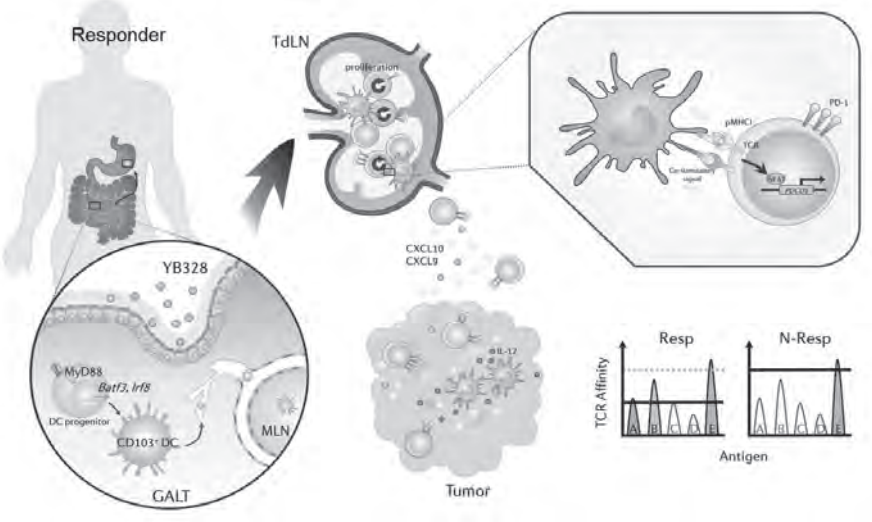
5) YB328-induced CD103$^+$ DC migration from the gut to lymphoid organs and tumors

We examined the systemic distribution and migration dynamics of CD103$^+$ DCs induced by YB328. Using FCM, we observed significantly increased infiltration of CD103$^+$ DCs in the lamina propria, Peyer's patches, draining lymph nodes (dLNs), and tumors. In contrast, no increase in CD103$^-$ myeloid cells was observed. To trace the migration route, we employed Kikume-GR mice, in which cells convert from green (KikG$^+$) to red (KikR$^+$) upon local UV exposure. Following UV illumination of the intestinal region and oral YB328 administration, we detected KikR$^+$ CD103$^+$ DCs in dLNs and tumors. These findings indicate that YB328-activated DCs originate in the gut and migrate systemically to modulate immune responses at tumor sites.

6) Clinical validation: Correlation of YB328 colonization with immune infiltration in human tumors

To validate our findings in human cancer, we analyzed pretreatment tumor samples from patients included in our cohort using multiplex IHC. CD103$^+$ DCs in mice correspond to CLEC9A$^+$ IRF8$^+$ DCs in humans. Integrated analysis of microbiota composition and multiplex IHC revealed that patients harboring YB328 exhibited significantly higher intratumoral infiltration of PD-1$^+$ CD8$^+$ T cells and CLEC9A$^+$ IRF8$^+$ DCs. These results indicate that the YB328-driven immunological mechanisms observed in mice are conserved in human tumors, suggesting its translational potential for enhancing cancer immunotherapy.

Graphical summary



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METABOLIC TRIGGERS DURING CANCER EVOLUTION

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Cancer cells arising in many tissues differ significantly in their functional capabilities from normal cells in the same tissue. These functional differences – termed 'cancer hallmarks' - are shared generally across many cancer types. They enable cancer cells to escape the bounds which normally limit cell behaviour, initiating and promoting carcinogenesis. Emerging evidence suggests that metabolic alterations in cancer cells and their microenvironment may promote or enable carcinogenesis in this way. Multiple metabolic stresses occur during carcinogenesis and cancer progression in many different tissues. In response, cancer cells undergo metabolic reprogramming, which supports their proliferation and survival at different points in their evolution. Many different changes have been reported, including increased aerobic glycolysis (the Warburg effect), altered amino acid metabolism, increased reactive oxygen species (ROS), hypoxia and nutrient deprivation. In particular, glycolysis, a fundamental pathway for energy metabolism, is deeply implicated in carcinogenesis. Cancer cells typically rely on aerobic glycolysis to adapt to the metabolic demands of macromolecule synthesis for cell proliferation, or in response to other stimuli. Known oncogenic alterations like KRAS mutations promote aerobic glycolysis, as does adaptation to tumor hypoxia via the von Hippel-Lindau pathway, which promotes glycolysis relative to oxidative phosphorylation (OxPhos).

Knudson's 'two hit' paradigm posits that carcinogenesis requires inactivation of both copies of an autosomal tumor suppressor gene. We have recently reported (1) that the glycolytic metabolite, methylglyoxal (MGO), transiently bypasses Knudson's paradigm by inactivating the breast cancer suppressor protein, BRCA2, to elicit a cancer-associated, mutational single-base substitution (SBS) signature in non-malignant mammary cells or

patient-derived organoids. Exogenously added MGO, or intracellular MGO generated endogenously by cancer-associated anomalies in OxPhos or mitochondrial metabolism that elevate glycolysis, induces BRCA2 proteolysis, disabling its functions in DNA repair and replication. Cells carrying monoallelic germline BRCA2 truncations exhibit greater sensitivity than wild-type cells to MGO-induced BRCA2 degradation and its consequences. Metabolite-induced BRCA2 inactivation in this setting is transient, in that functionally adequate levels of BRCA2 expression are later recovered. Nevertheless, it is sufficient to temporarily impart a mutator phenotype. Similar changes occur in a genetically engineered, autochthonous murine model for mutant KRAS-driven tissue-specific pancreatic carcinogenesis provoked by BRCA2 inactivation, and in a subset of human breast cancers. Intermittent exposure to MGO over prolonged periods suffices to provoke episodes of genome-wide SBS mutagenesis, without permanent inactivation of BRCA2 expression or activity, promoting cancer genome evolution. Collectively, our findings reveal a mechanism whereby endogenous or environmental factors that alter glucose metabolism could bypass the Knudson 'two hit' requirement via MGO-induced BRCA2 haploinsufficiency, to promote episodes of cancer genome evolution that initiate or sustain tumorigenesis. These findings could link glycolysis activation by oncogenes, metabolic disorders, or dietary challenges, to mutational signatures implicated in cancer evolution (**Figure 1**).

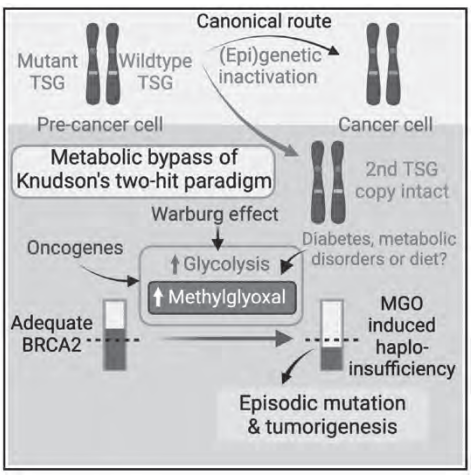

Figure 1 Cells carrying a monoallelic germline BRCA2 mutation can bypass Knudson's "two-hit" requirement to initiate tumorigenesis when the glycolytic metabolite methylglyoxal transiently disables BRCA2 function. Metabolic bypass of tumor suppressor activity may link metabolic reprogramming, metabolic disorders, or diet to early steps in carcinogenesis.

Whilst our above-noted observations highlight how host metabolism may alter cancer progression, this relationship is also reciprocal. For example, cancer can alter host metabolism by inducing profound changes in nutrient intake and handling that culminate in cachexia. Cancer cachexia is a severe wasting syndrome that is characterized by reduced food intake and terminal weight loss that affects up to 80% of all patients with cancer, and

causes significant morbidity and mortality. This persistent metabolic stress condition increases glucocorticoid levels in humans and mouse models of cancer cachexia.

Cancer growth has been targeted by the utilization of ketogenic diets (KDs) comprising high-fat and low-carbohydrate levels, which leverage the dependency of cancer cells on glucose. However, the effect of KDs on the host organism – besides the tumor itself – is poorly studied. We have determined the differential effect of KD on tumors and the host organism using two murine models of cancer cachexia (2). Paradoxically, we find that although KD slows tumor growth, it shortens survival by accelerating cachexia. Mechanistically, increased lipid peroxidation in KD-fed tumor-bearing mice leads to systemic redox imbalance. Within tumors, this results in saturation of the GSH pathway, formation of lipid peroxidation produts (LPPs), and consequent ferroptotic death of cancer cells. Moreover, we find in mice fed KD, that NADPH depletion impairs corticosterone biosynthesis in the adrenal cortex, inducing a relative adrenal insufficiency and metabolic maladaptation. Treatment with dexamethasone delays the onset of cancer cachexia and extends survival of tumor-bearing mice fed KD by improving food intake, metabolic homeostasis, and utilization of nutritional substrates while preserving the anti-tumor response (**Figure 2**). Thus, or findings uncouple the effects of KD on tumor growth from overall survival of the host, illustrating the reciprocal relationship between cancer and host metabolism.

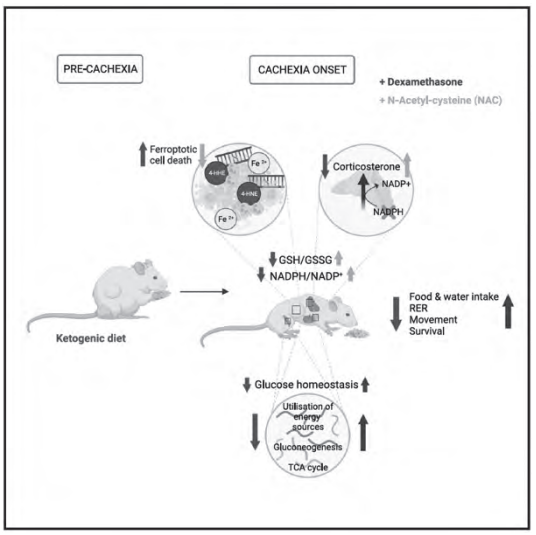

Figure 2 The anti-cancer effects of a ketogenic diet are uncoupled from survival in mouse models of IL-6-producing cancers. Intratumoral ferroptosis causes a smaller tumor burden, but systemic NADPH depletion induces relative hypocorticosteronemia, which accelerates cachexia onset.

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INTERPLAY WITH MICROENVIRONMENT PROVOKES TUMOR FORMATION IN THE KIDNEY

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Sustained genome/epigenome damage caused by, for instance, chronic inflammation or exposure to mutagens is considered to raise a risk for cancer development. A variety of human conditions give rise to these cancer-prone tissues such as chronic hepatitis with HBV/HCV infection and chronic gastritis with *Helicobacter Pylori*.

Damaged kidneys under long-term hemodialysis (HD) are another example for cancer-prone tissues. Kidneys regulate water/ion balance of human bodies, and a severe attenuation of renal function requires the treatment of HD or kidney transplantation. While HD can compensate for normal renal function to some extents, a long time HD-treatment almost always leads to acquired cystic kidney disease (ACKD)[1]. Kidneys under such condition are filled with micro- to small-cysts, and, more importantly, have a significantly increased risk for renal cell carcinoma (RCC). Majority of ACKD-associated RCC are not clear cell RCC (ccRCC) that is a predominant cancer type in sporadic RCC, but has a variety of pathological subtypes[2]. Patients dependent on dialysis are more than 500,000 in U.S.A. (https://esrdnetworks.org/resources-news/national-esrd-census-data/) and more than 300,000 in Japan[3]. So ACKD is a huge burden for human well-being worldwide.

The mechanism of cyst formation and subsequent tumor evolution under ACKD is totally unknown yet. To gain insights into such cancer trajectory, we analyzed the genomic/transcriptomic profiles of cystic- and normal-cortex in ACKD- and normal-kidneys, respectively, and also of various RCCs. Mutations within *VHL* that are the hallmark of ccRCC were not identified in ACKD-related RCC. Interestingly, the transcriptomic profile of cystic and malfunctioning cortex of kidneys is distinct from that of normal cortex, mainly separated by the expression profile of immune-related genes. Further, single-cell and spatial

transcriptomic analyses of these specimens revealed that proximal tubule (PT) cells are degenerated into damaged terminal PT cells in ACKD, but a minor fraction of such PT cells become transformed into cystic cells (Figure 1). Such transformation is shown supported by the paracrine activation of MET tyrosine kinase with its ligand, hepatocyte growth factor, produced from fibroblasts adjacent to cyst cells[4].

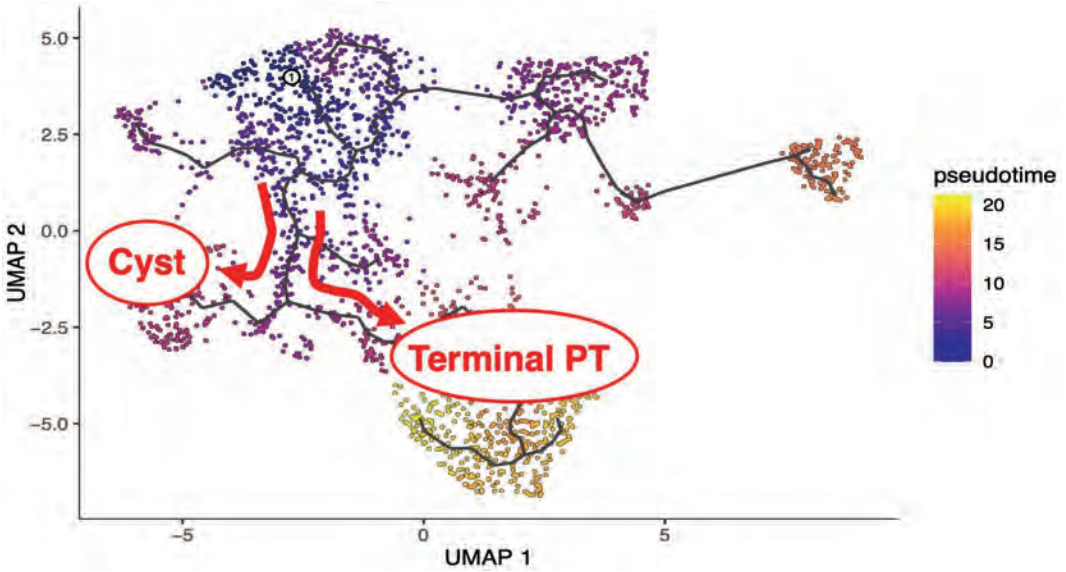

Figure 1 Trajectory of proximal tubule cells in ACKD

Single-cell analyses on proximal tubule (PT) cells in normal kidney and ACKD reveal that normal PT cells become degraded into terminal PT ones, but a fraction of them are transformed into cysts.

Surprisingly, exome sequencing of cyst cells isolated with laser microdissection has evidenced that every cyst has somatic gene mutations with a very high variant allele frequency (VAF) of 10-50%. Frequently affected genes included those for the cohesin complex and histone modifiers. These lines of evidence indicate that cysts result from mono- or oligo-clonal growth of PT cells carrying somatic mutations at cohesin/histone modifier genes, not from balloon-like enlargement of PT structure due to urine retention. Combination of cohesin mutations and MET activation may be an underlying mechanism for cyst formation. Through the genomic analyses of the cystic and RCC cells, it became evident that the latter cells are generated from the former cell clone. Interestingly, ACKD-associated RCC has a distinct mutation profile from sporadic RCC (mainly ccRCC).

Further, ACKD and its associated RCC have each specific profile of immune cells in the kidney, and, importantly, both M1- and M2-macrophages drastically increased upon tumor formation. Single cell analyses reveal subsets of macrophages have changed in the tumor

microenvironment during the step of malignant transformation; both ISG15$^+$ and SPP1$^+$ macrophages drastically increased specifically upon tumor formation. Such increase of SPP1$^+$ macrophages has been reported also in colorectal[5] and head and neck cancers[6], and specific inflammation profiles seemed to nourish the pro-tumor condition. A variety of interplay with immune cells in the kidney triggers the growth of multiple cysts and, eventually, of tumors from such cysts.

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PERSONALIZED CANCER VACCINES: UPDATES AND PROMISING RESULTS

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Personalized neoantigen-targeting vaccines can induce durable and diverse T cell responses and have been associated with improved outcomes. In early clinical studies, genomics-guided personalized cancer vaccines (PCVs) have demonstrated the capabilities of inducing long-term, tumor-specific immune responses across malignancies, clinical settings, and treatment regimens. Now that PCVs have advanced to large-scale, randomized clinical trials, we are at a pivotal time.

Investigator-initiated trials have a critical place in the lifecycle of translating foundational basic science findings to clinical practice. They provide the opportunity to perform that essential 'human experiment', in which a new idea is tested, and they serve as a platform from which to consistently collect informative biospecimens at key time points relative to the treatment intervention, so that we can interrogate the key questions that can provide mechanistic insight regarding the impact of our intervention. Importantly, such inquiry provides a springboard from which new ideas for how to innovate the space can take flight. Indeed, the future success of PCVs will be likely dictated by our collective ability to apply and iterate upon the foundational lessons gained from early and ongoing intensive in-depth studies so that we can rationally exploit the cytolytic capabilities of PCVs. In one recent example, we conducted a phase I trial of neoantigen-targeting PCVs in patients with high-risk, fully resected clear cell renal cell carcinoma (RCC; stage III or IV), with or without locally administered ipilimumab (*Braun Nature 2025*). At a median follow-up of 40.2 months from surgery, none of the 9 initial participants experienced a recurrence of RCC. No dose-limiting toxicities were observed. Our results demonstrate that neoantigen-targeting PCVs in

high- risk RCC are highly immunogenic, capable of targeting key driver mutations, and induce anti-tumor immunity. These observations, in conjunction with the early signal of clinical activity, highlights the promise of PCV as effective adjuvant therapy following total resection for high-risk disease. In another recent study, we tested the impact of formulating a synthetic long peptide vaccine with the adjuvants Montanide and poly-ICLC in 10 patients with melanoma (*Blass & Keskin Cell 2025*). Hundreds of circulating and intratumoral T cell receptor (TCR) clonotypes emerged following vaccination that were distinct from those arising after PD-1 inhibition. By linking vaccine-specificity and cellular phenotype of TCRs in post-treatment tumors at single cell resolution, we demonstrated remodeling of the intratumoral T cell repertoire following vaccination. These observations support the impact of multi-pronged immune adjuvanticity on the vaccine-induced T cell repertoire in the context of neoantigen-targeting vaccines.

A multitude of ongoing questions remain under active investigation. How best can we deliver vaccines and what are rational combinations of immunomodulatory agents in relationship to individual tumor microenvironments? Which class of immune checkpoint inhibitors optimizes the anti-tumor efficacy of PCVs? What mechanisms drive immune evasion in disease progression? Are there alternative tumor-specific antigen targets to discover? How can we personalize treatment regimens to address intratumoral heterogeneity? As we look ahead to the results of pivotal clinical trials, intensive investigative efforts are needed to elucidate optimal treatment sequences, vaccine adjuncts and delivery mechanisms, biomarker development, improved epitope prediction algorithms, and improvements in vaccine manufacturing efficiency. While these trial results will determine the short-term impact of PCVs, their long-term success depends on collaborative efforts to apply insights from first-generation trials. We are hopeful to leverage the capabilities of PCVs to reduce cancer-associated morbidity and mortality by eradicating cancer in the metastatic setting, curing patients in the post-operative setting, and intercepting oncogenesis in high-risk patients.

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TUMOR-INFILTRATING PLASMA CELLS AND HUMORAL RESPONSES TO CANCER-TESTIS ANTIGENS UNDERPIN CLINICAL BENEFIT TO IMMUNE CHECKPOINT BLOCKADE

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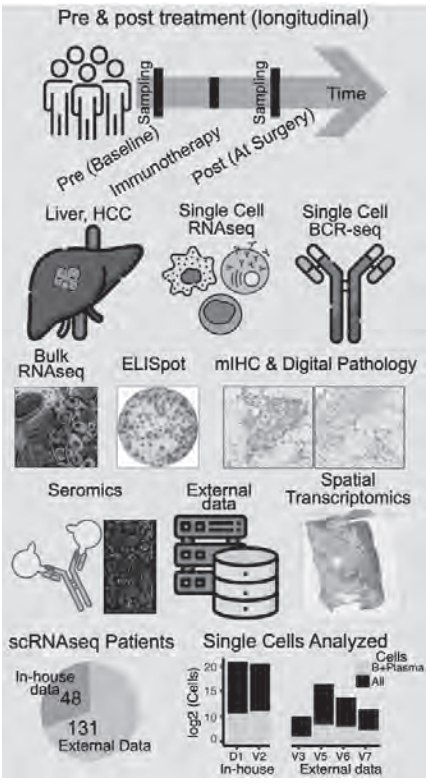
Identifying immune signatures that predict response to immune checkpoint blockade is the key to guiding appropriate treatment to patients and to providing insights into mechanisms of therapeutic resistance to immunotherapy. The list of currently approved biomarkers (PD-L1 expression, mutation burden) needs expansion through coordinated efforts to rigorously assess tissue, cellular and humoral immune signatures.



Tumor-infiltrating T cells have been the primary focus of cancer immunotherapy, with ample data showing that they are associated with clinical benefit in various tumors. However, accumulating evidence points to a critical role for B cells and plasma cells in shaping responses to immune-checkpoint blockade. We investigated the humoral immune response in hepatocellular carcinoma patients treated with neoadjuvant anti–PD-1 therapy. In responders, defined by >50% tumor necrosis, we observed on-treatment enrichment of clonally expanded $IgG1^+$ plasma cells within the tumor, using single-cell and bulk RNA sequencing. This was confirmed in tissues stained by multiplex immunohistochemistry which

were analyzed using a newly developed pipeline named MARQO that quantifies whole slides at the single-cell level (1). Clonal tracking revealed that anti–PD-1 treatment expanded pre-existing B cell clones, which were associated with favorable clinical outcomes. These findings were validated in multiple independent cohorts receiving PD-1 and VEGFA blockade.

Moreover, serum from responders contained IgG1 antibodies specific to cancer-testis antigens (Figure 1), including NY-ESO-1, and these humoral responses were linked to tumor-reactive T cell activity. Previous work from the lab demonstrated that such antibodies can form immune complexes and facilitate cross-presentation of antigen to T cells (2), that they are enriched in melanoma patients responding to ipilimumab (3), and are also significantly associated with better survival in endometrial cancer patients receiving combination treatment of nivolumab and cabozantinib (4).

Collectively, our results demonstrate that PD-1 blockade induces tumor-specific $IgG1^+$plasma cell responses that complement cellular immunity and contribute to clinical benefit, underscoring a coordinated humoral-cellular axis in effective anti-tumor immunity.

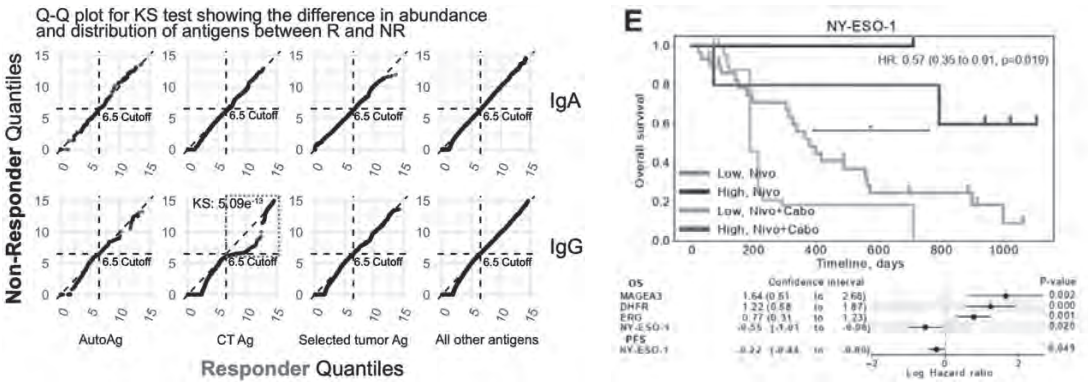


Figure 1 Left: Enrichment in IgG reactivity to cancer testis antigens (CT Ag) in hepatocellular cancer patients responding to neoadjuvant PD-1 blockade. Right: Advanced endometrial patients with NY-ESO-1 serum antibodies at baseline have improved survival following combination of nivolumab with cabozantinib.

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MICROENVIRONMENTAL NICHES SUPPORTING MALIGNANT LYMPHOMA

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Malignant lymphomas (MLs) are blood cancers in which tumor cells mimic mature lymphocytes. MLs are broadly categorized into B-cell and T/NK-cell neoplasms based on the lineage of the tumor cells, and are further classified into approximately 100 distinct entities according to a combination of histopathological features, immunophenotypic profiles, clinical course, and underlying genomic alterations.

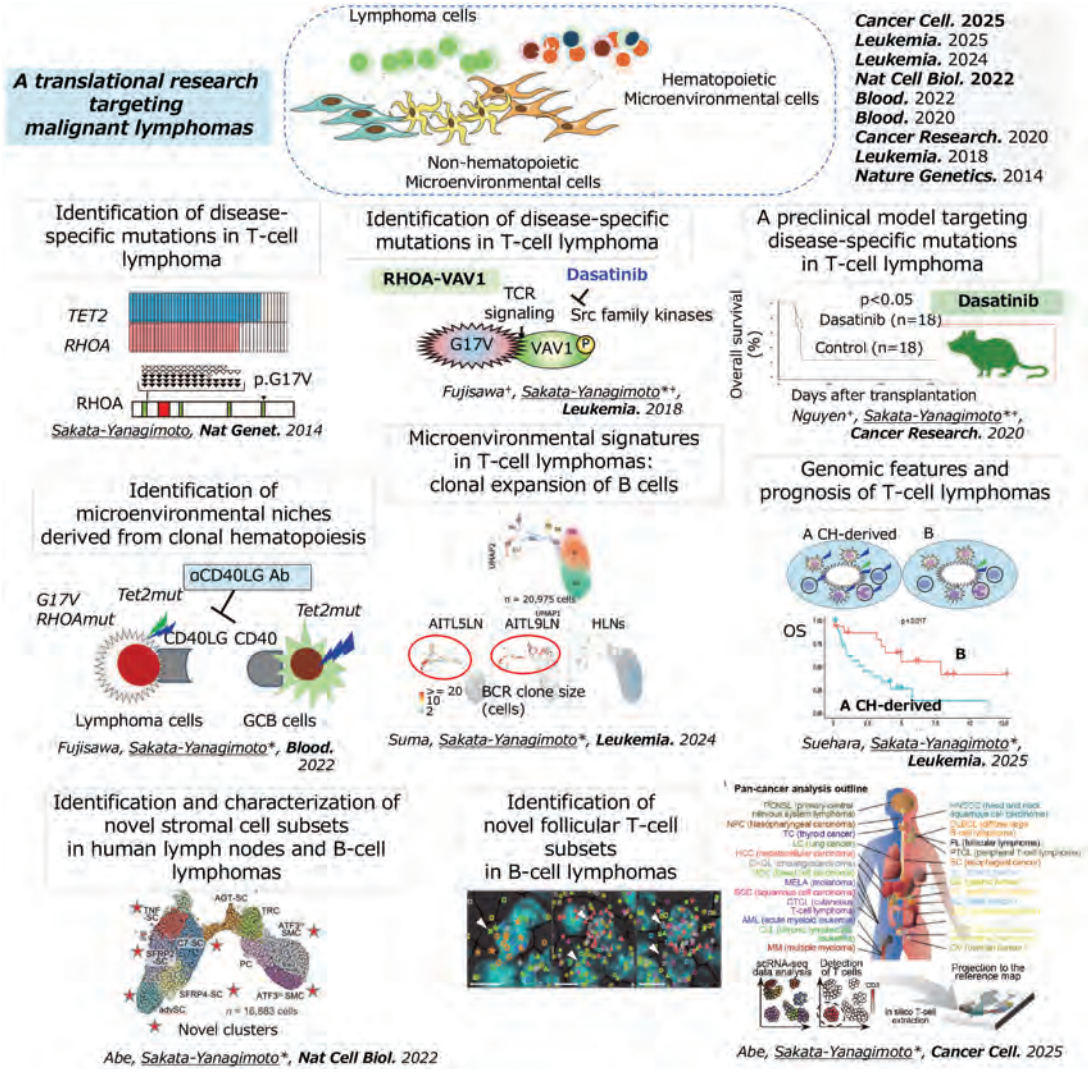
Understanding the pathogenesis of MLs requires not only deciphering the intrinsic abnormalities of tumor cells but also elucidating the tumor-supportive roles of the surrounding microenvironment. Our research has focused on the immune microenvironment derived from clonal hematopoiesis (CH), and the ecosystem formed by immune and stromal components, with the aim of uncovering disease-specific microenvironmental niches.

I. Microenvironmental immune cells derived from clonal hematopoiesis in angioimmunoblastic T-cell lymphoma (AITL).

AITL is a rare and aggressive subtype of peripheral T-cell lymphoma (PTCL) characterized by diagnostic difficulty and poor prognosis, with a 5-year overall survival rate of approximately 30%. Our group has extensively investigated the genetic and microenvironmental landscape of AITL, aiming to establish novel diagnostic and therapeutic strategies.

(i) Identification of Recurrent Genomic Alterations in AITL (*Nat Genet.* 2014)

By using extensive genomic analyses, we identified that up to 70% of AITL cases harbor



the recurrent somatic mutation, p.Gly17Val (G17V) *RHOA*, encoding a small GTPase. Notably, this mutation was almost exclusively observed in AITL and was accompanied by loss-of-function mutations in *TET2*, encoding an epigenetic regulator. These mutations were found to co-exist in tumor cells, with G17V restricted to T-lineage tumor cells, while *TET2* mutations were detected in both tumor cells and various inflammatory immune cells in the tumor microenvironment (***Nat Genet.* 2014**). These findings led us to propose a multistep lymphomagenesis model: During the aging process, *TET2* mutations are acquired in hematopoietic stem cells, giving rise to premalignant cells. These cells correspond to what has been simultaneously described as "clonal hematopoiesis (CH)" by international research groups (Genovese G., ***NEJM***. 2014; Jaiswal S., ***NEJM***. 2014). The subsequent acquisition of

G17V in premalignant cells provokes malignant transformation towards T-lineage tumor cells. This model highlights the clonal evolution from CH to overt lymphoma, providing a new conceptual framework for the origin of AITL ("Multistep tumorigenesis in the AITL development").

Given the high specificity of G17V for AITL, we developed a sensitive and efficient method to detect this mutation in clinical samples, including lymph node biopsies, pleural effusions, and peripheral blood. This diagnostic assay was validated and subsequently patented, and the technology was transferred to industry. The method allows for rapid molecular confirmation of AITL and can assist in challenging diagnostic cases, underscoring the utility of genomics in clinical hematopathology.

(ii) Discovery of the RHOA-VAV1 Signaling Axis and Targeting Therapies (*Leukemia*. 2018, *Blood*. 2020, *Cancer Research*. 2020).

Through comprehensive proteomic analyses, we identified VAV1—a critical mediator of T-cell receptor (TCR) signaling—as a direct binding partner of the G17V RHOA mutant. VAV1 typically exists in an autoinhibited state, but binding with G17V-RHOA enhances its phosphorylation and activates downstream TCR signaling. Interestingly, in G17V-negative AITL cases, gain-of-function mutations in *VAV1* were detected, suggesting hyperactivation of TCR signaling in these cases. These findings established the "RHOA-VAV1 axis" as a central driver in AITL pathogenesis (***Leukemia*. 2018, *Blood*. 2020**). We further demonstrated that this pathway can be effectively inhibited by dasatinib, a multi-kinase inhibitor approved for Philadelphia chromosome-positive leukemia. Oral administration of dasatinib significantly prolonged survival in genetically engineered AITL model mice. Subsequently, we conducted a phase I clinical trial to assess the safety profile in patients with relapsed/refractory AITL, translating our benchside findings into a clinical setting (***Cancer Research*. 2020**)). We further performed an investigator-initiated clinical study evaluating dasatinib in patients with relapsed/refractory AITL and its related lymphomas.

(iii) Functional Role of *TET2*-Mutated Inflammatory Cells in the Tumor Microenvironment (*Blood*. 2022, *Leukemia*. 2024, *Leukemia*. 2025)

These studies prompted a key question: ***why does AITL arise through a multistep tumorigenic process originating from such premalignant cells***? To address this, we hypothesized that not only the tumor cells themselves but also immune cells derived from the premalignant cells in CH status contribute to the formation of a supportive microenvironmental niche that promotes tumor development. By combining single-cell analysis and functional studies using both patient-derived samples and mouse models, we revealed that *TET2*-mutant immune cells infiltrate the AITL tumor microenvironment and

support tumor growth. Specifically, germinal center B (GCB) cells with *TET2* mutations acted as a supportive niche by engaging in CD40-CD40LG interactions with tumor T cells. This interaction promoted tumor cell survival and proliferation (***Blood*. 2022**). Moreover, we characterized a distinctive immune exhaustion signature in the AITL microenvironment, which may be related to therapeutic resistance in AITL patients (***Leukemia*. 2024**). Importantly, genomic alterations were found to correlate with the composition and functional states of the microenvironment as well as tumor cells, and were associated with patients' survival (***Leukemia*. 2025**). These insights support the notion that AITL development is not solely tumor cell-autonomous but highly influenced by CH-derived immune cells and their interactions within the niche.

II. Microenvironmental ecosystem in Follicular Lymphoma (FL) (*Cancer Cell*. 2025, *Nat Cell Biol*. 2022).

FL is an indolent B-cell lymphoma with a highly variable clinical course. While patients with high tumor burden typically undergo immunochemotherapy, those with low tumor burden are often managed by watchful waiting. Intriguingly, a subset of patients experiences spontaneous tumor regression during observation, implying a pivotal role of the tumor microenvironment in disease modulation in FL. To elucidate the underlying mechanisms, we established a single-cell analytical platform for dissecting the FL stromal microenvironment. We constructed a comprehensive single-cell atlas of non-hematopoietic stromal cells, including vascular, lymphatic, and non-endothelial lineages, and identified transcriptional changes specific to FL-associated stromal populations compared to reactive lymph nodes. Computational inference of ligand-receptor interactions revealed novel points of crosstalk between tumor B cells and the stromal compartment (***Nat Cell Biol*. 2022**).

Furthermore, we performed single-cell and spatial transcriptomic analyses of tumor-infiltrating T cells in FL. This approach led to the discovery of previously unrecognized follicular T-cell subsets, characterized by distinct gene expression patterns and localization within or near neoplastic follicles. Notably, the presence of this subset was associated with favorable clinical outcomes. Given its prognostic relevance, we have filed a patent application for its potential use as a biomarker (***Cancer Cell*. 2025**).

III. Conclusion and Future Directions.

Our studies demonstrate that genomic alterations in both tumor and non-tumor cells are intimately linked to the formation of lymphoma-specific microenvironments. In AITL, we have defined a paradigm in which premalignant status of CH gives rise to both tumor cells and supportive niche cells, driving disease through cooperative interactions. In FL, we have uncovered a complex stromal and immune landscape that shapes disease progression and

may hold the key to understanding spontaneous remission. These findings provide a strong foundation for biomarker development and the design of mechanism-based, niche-targeted therapies. We will continue our research to unravel the eco-evolutionary dynamics and pave the way toward precision immuno-oncology in MLs.

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THE DISCOVERY OF MASTER SWITCH OF REGULATORY T CELLS AND ITS THERAPEUTIC TARGETTING FOR CANCER AND AUTOIMMUNE DISEASES

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Regulatory T cells (Tregs) are an important immune subtype indispensable for immune homeostasis. The primary role of Tregs is maintaining self-tolerance by dampening aberrant or excessive immune reactions to healthy tissues and/or innocuous environmental stimuli. Treg defects, including reduced numbers, disrupted immunosuppressive capabilities, and acquisition proinflammatory phenotypes underpin the etiology of various autoimmune diseases, such as psoriasis, type 1 diabetes, rheumatoid arthritis, ulcerative colitis, multiple sclerosis, Sjögren's Disease, systemic lupus erythematosus, myasthenia gravis, and systemic sclerosis. In contrast, intratumoral Tregs suppress antitumor immune responses, thus enabling tumors to thrive. Accordingly, a high ratio of Tregs to $CD8^+$ T cells predicts poor prognosis in a variety of tumor types, including ovarian cancer, pancreatic ductal adenocarcinoma, triple-negative breast cancer, and hepatocellular carcinoma. Therapeutic targeting of Tregs could therefore address the root of these diverse diseases and potentially improve disease outcomes.

Efforts to modulate Tregs have suffered from poor targets. Cell surface targets often lack specificity, which can produce off-target effector immune cell modulation and hamper efficacy. Targeting downstream effector molecules is potentially a more specific approach; however, this does not address the full range of Treg function. To overcome these challenges, we first sought to identify a specific controller of Treg phenotype and function that could be targeted therapeutically. We identified a novel, specific Master Switch of Tregs. We found that this Master Switch is responsible on the fully activity of T regulatory cells in the immune system; It controls the suppressive function of nTregs and controls the secretion of the immunosuppressive cytokines TGFβ and IL-10 and fully regulates the ability of $CD4^+$ T cells

to convert into iTregs in the presence of TGFβ by regulating the production of stability of FOX03. Thus, Master Switch i) regulates Treg suppressive activity; ii) controls *de novo* Treg induction of from $CD4^+$ T cells; and iii) stabilizes FOXP3 (Figure 1). We further found that this Master Switch is not functional in non-Treg effector T cell function or has any known function in other type of cells. It is therefore an ideal therapeutic target of Tregs.

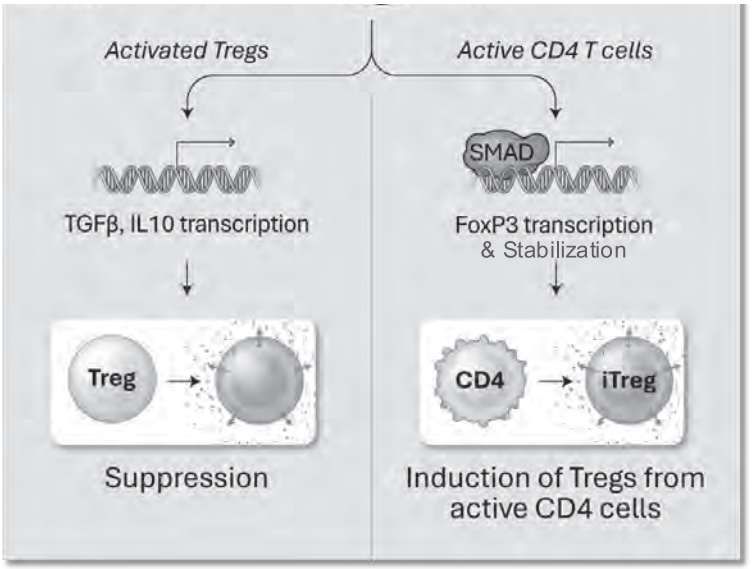

Figure 1 Mechanism of Regulatory T Cell Control by Master Switch

Because of the crucial role of Master Switch in regulating Treg cells, we developed the first-in-class small-molecule (SM) selective Treg activator and selective Treg inhibitor of Master Switch. The "SM activator" enhances the suppressive function of existing Tregs and increases the conversion of activated $CD4^+$ T cells into Tregs. We found that this activator demonstrated therapeutic efficacy across multiple models of inflammatory disease, and currently being tested in early-stage clinical trials. On the other hand, SM Treg inhibitor prevents Treg induction from non-Treg $CD4^+$ cells and inhibits the suppressive function of existing Tregs. The loss of Treg-mediated immunosuppression in tumors corresponds with increased effector T cells and reduced tumor growth across multiple murine solid tumor models. It is also being tested in phase 2 clinical trial with multiple indications.

The novel biology and the role of the drugs will be presented

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THE ROLE OF MITOCHONDRIAL METABOLISM IN CANCER IMMUNITY

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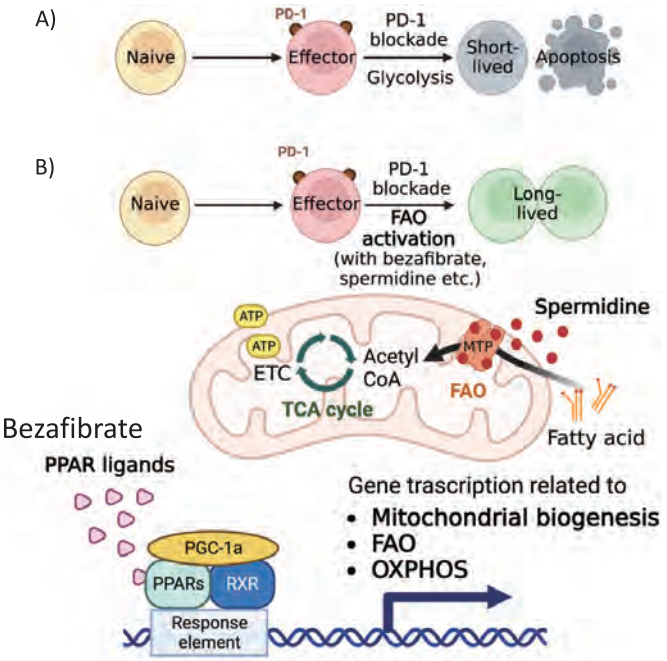
Immune checkpoint inhibitor (ICI) therapy, particularly using PD-1 antibodies, has become a cornerstone in the treatment of a wide range of cancers. Despite its broad application, more than half of the patients remain unresponsive to this therapy. Since $CD8^+$ T cells are the primary effectors of anti-tumor immunity, restoring their function in the tumor microenvironment (TME) is crucial for enhancing therapeutic outcomes. In our research, we have focused on the role of mitochondrial metabolism in regulating $CD8^+$ T cell function, particularly through mitochondrial fatty acid oxidation (FAO).

We found that tumor-infiltrating $CD8^+$ T cells in unresponsive tumors exhibited impaired mitochondrial function in mice [1]. This dysfunction of mitochondrial metabolism can be ameliorated by small-molecule compounds such as bezafibrate and spermidine, both of which enhance mitochondrial FAO.

Bezafibrate, a PPAR agonist, upregulates CPT1a and stabilizes the anti-apoptotic protein Bcl-2 in $CD8^+$ T cells, thereby promoting their survival within the tumor. In murine models, bezafibrate combined with anti-PD-1 therapy increased T cell persistence and suppressed apoptosis, leading to enhanced anti-tumor effects [2]. In a Phase I clinical trial involving EGFR mutation-negative NSCLC patients, bezafibrate combined with nivolumab prolonged progression-free survival (PFS) compared to nivolumab alone [3]. The combination therapy elevated systemic FAO markers in plasma and improved mitochondrial metabolism and effector function in peripheral $CD8^+$ T cells in the patients.

Spermidine, a bioactive polyamine, is recognized for its anti-aging properties. In the context of immune aging, where anti-tumor immunity is impaired, we investigated the role of spermidine. Spermidine levels naturally decline with age in $CD8^+$ T cells, leading to

compromised mitochondrial FAO and diminished T cell function. We demonstrated that spermidine binds directly to the mitochondrial trifunctional protein, a key FAO enzyme, thereby enhancing FAO activity and restoring $CD8^+$ T cell anti-tumor responses in aged mice [4]. Exogenous administration of spermidine reversed resistance to PD-1 blockade therapy in aged mice, offering a compelling avenue for rejuvenating aged immunity. We screened and identified spermidine analogue for the clinical application in the future [5]. These findings indicate that mitochondrial FAO is a critical determinant of T cell resilience and response to checkpoint blockade. Targeting mitochondrial metabolism may represent a promising next-generation strategy for improving immunotherapy efficacy (Fig. 1) [6].



Based on Chamoto K et al. ***Nat Rev Immunol***, 23:682, 2023

Fig.1 Mitochondrial FAO changes the phenotype of CD8+ T cells in TME.

The remaining question in our concept is how FAO in T cells is important for enhancing anti-tumor immunity. A crucial aspect of T cell dysfunction in cancer is oxidative stress–induced metabolic disorder, particularly through the accumulation of reactive oxygen species (ROS) and lipid peroxidation byproducts. We found that FAO is critical for attenuating extra-oxidative stress and preventing the exhaustion process in $CD8^+$ T cells within the TME.

In tumor-infiltrating T cells, we identified a distinct form of metabolic exhaustion characterized by increased glycolysis and suppressed FAO—opposite to the metabolic

profile typically associated with PD-1 signaling. We termed this phenotype "metabolic exhaustion." Single-cell analyses and mitochondrial profiling revealed that the most exhausted $CD8^+$ T cells exhibited elevated mitochondrial membrane potential but reduced FAO enzyme expression. These cells accumulated lipid peroxidation end-products, particularly active aldehydes such as acrolein [7].

Active aldehydes are significantly more reactive and cytotoxic than conventional ROS. Using phospho-proteomics and electron microscopy, we found that acrolein disrupts mitochondrial cristae architecture and impairs FAO within T cells, while simultaneously activating mTORC1 signaling and glycolysis. These data suggest a deleterious feedback loop in which FAO impairment amplifies aldehyde production, further exacerbating mitochondrial damage and T cell exhaustion. Indeed, T cell-specific deletion of FAO enzymes led to increased aldehyde accumulation and severe exhaustion in $CD8^+$ T cells in TME, resulting in diminished anti-tumor immunity in vivo. Super-resolution microscopy confirmed that aldehyde production occurs, at least in part, within the T cell mitochondria themselves, damaging mitochondrial membrane immediately.

Collectively, our findings propose a model as show in in Fig. 2 in which:

1. T cell activation triggers low-level mitochondrial lipid peroxidation, leading to the accumulation of toxic aldehydes, including acrolein.
2. These aldehydes directly suppress FAO and reinforce glycolytic bias via mTORC1 activation.
3. The resulting suppression of FAO further accelerates aldehyde production, boosting metabolic alterations and T cell exhaustion.

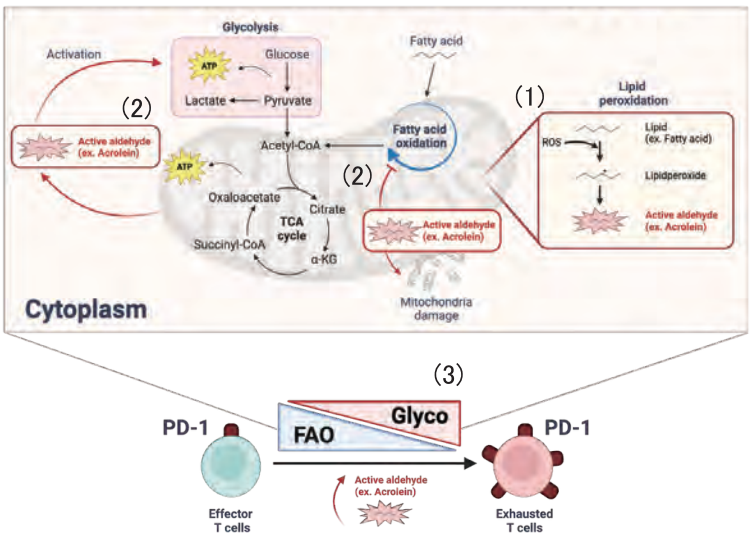


Fig. 2 Negative loop toward metabolic exhaustion by active aldehydes.

This cascade of oxidative stress, aldehyde accumulation, and mitochondrial collapse provides a compelling explanation for the progressive dysfunction of tumor-infiltrating T cells. Importantly, pharmacologic scavenging of aldehydes or enhancement of FAO represents a viable strategy for breaking this cycle and restoring T cell efficacy within tumors [7].

In conclusion, our research highlights the pivotal role of mitochondrial metabolism, specifically FAO, in determining the fate and function of $CD8^+$ T cells in cancer immunity. Robust FAO activity prevents T cells from damage by oxidative stress; these metabolic analyses serve as both biomarkers of ICI efficacy and actionable therapeutic targets. Enhancing mitochondrial FAO and mitigating oxidative stress may thus unlock new strategies for potentiating ICI and overcoming resistance in cancer immunotherapy.

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AGING AND CANCER: NOVEL CONNECTIONS

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Aging and cancer are characterized by partially overlapping hallmarks. Thus, several hallmarks of aging (i.e., genomic instability, epigenetic alterations, chronic inflammation, and dysbiosis) clearly contribute to oncogenesis and tumor progression and hence are also considered as hallmarks of cancer. Disabled autophagy and cellular senescence are two hallmarks of aging that exert context-dependent oncosuppressive and pro-tumorigenic effects as well, likely explaining the fact that organismal aging is linked to an increase in the likelihood of developing malignant disease. Beyond the concept of hallmarks, cancer and aging can also be interpreted as the result of alterations in genes that are referred to as oncogenes (for cancer) and gerogenes (for aging).

We recently identified a new mechanistic connection between aging and cancer that is mediated by the autophagy-inhibitory neuroendocrine factor acyl CoA binding protein (ACBP), which is encoded by the gene *diazepam binding inhibitor* (DBI). ACBP/DBI is a small (88 amino acids), evolutionarily conserved protein that binds intracellular lipids, including medium- and long-chain acyl-CoA esters, thereby regulating their metabolism in a wide range of cell types. Thus, intracellular ACBP/DBI favors fatty acid oxidation in glioblastoma and bone metastases of breast and lung cancer. Beyond its cell-autonomous metabolic role, ACBP/DBI functions as an extracellular signaling molecule that is secreted in response to autophagy induction or passively released in the context of cell death.

Once in the extracellular space, ACBP/DBI acts through paracrine or autocrine mechanisms, engaging γ-aminobutyric acid (GABA) type A receptors to modulate cell fate

and inflammation. Intriguingly, ACBP/DBI appears to form part of a feedback loop that limits autophagic activity: while autophagy promotes its secretion, extracellular ACBP/DBI in turn suppresses autophagy, thus serving as a brake on prolonged catabolic activation or "autophagy checkpoint". Neutralization of extracellular ACBP/DBI—using antibodies, genetic ablation or receptor mutation—has been shown to exert potent cytoprotective, anti-inflammatory, anti-fibrotic, and tumor-suppressive effects in multiple preclinical models. These beneficial outcomes are at least in part attributable to the restoration of autophagic flux and the attenuation of pro-inflammatory signaling, positioning ACBP/DBI as a novel regulator at the interface of lipid metabolism, autophagy, and tissue homeostasis. Indeed, the effects of ACBP/DBI inhibition on healthspan are that pronounced, that ACBP/DBI has been classified as a pro-aging gene (product) or "gerogene". This idea is justified by the following facts:

First, in humans, ACBP/DBI plasma concentrations increase with chronological age in healthy individuals and further increase in the context of multiple different diseases including metabolic syndrome (correlating with glucose/insulin levels, dyslipidemia and blood pressure), cardiovascular disease and specific cancers including hepatocellular carcinoma. Thus, in relatively healthy centenarians, DBI/ACBP concentrations are approximately threefold higher than in younger (30-48 years) adults, but increase further upon hospitalization due to acute illness. More intriguingly, when an apparently healthy individual has higher ACBP/DBI levels than correspond to his/her age class, that individual is a particularly high risk of developing cardiovascular or malignant disease in the near future. Hence, high plasma ACBP/DBI concentrations may be considered as a risk facture for the future development of life-threatening age-related diseases.

Second, in model organisms, inhibition of ACBP/DBI delays aging. This applies to yeast (*Saccharomyces cerevisiae*), in which the knockout of the ACBP/DBI orthologue *ACB1* extends lifespan through an autophagy-dependent mechanism. It also applies to mice. In models of renal aging induced by cisplatin or doxorubicin, ACBP/DBI neutralization suppressed renal fibrosis and cellular senescence. In a model of hepatic aging induced by a combination of high-fat diet and carbon tetrachloride, ACBP/DBI inhibition prevented hepatocyte senescence and attenuated hepatosteatosis. Similarly, anti-ACBP/DBI reduced expression of the senescence marker cyclin-dependent kinase inhibitor 1A (CDKN1A, best known as p21) in cardiomyocytes from mice that were treated with DNA-damaging anthracyclines. Single-nucleus RNA sequencing of heart tissue revealed that anti-ACBP/DBI antibody restored key metabolic and cardioprotective gene expression patterns suppressed by doxorubicin. Accordingly, ACB/DBI neutralization suppressed the doxorubicin-induced decline of

cardiac function. Finally, ACB/DBI inhibition improved healthspan in a strain of progeroid mice.

Importantly, ACBP/DBI doe not only contribute to genera aging but may also participate to the pathogenesis of cancers. We observed that patients with cancer predisposition syndromes due to mutations in *BRCA1*, *BRCA2* or TP53 exhibit abnormally elevated plasma ACBP/DB I levels. In addition, patients without known cancer predisposition syndromes exhibit higher ACBP/DBI plasma concentrations before imminent cancer diagnosis (within 0-3 years) as compared to age and BMI-matched controls who remain cancer-free. Mouse experimentation revealed that genetic or antibody-mediated DBI/ACBP inhibition can delay the development or progression of cancers. This applies to hormone-induced breast cancer, as well as to urethane-induced non-small cell lung cancer. In the context of chemoimmunotherapy involving PD-1 blockade, DBI/ACBP neutralization enhances tumor infiltration by non-exhausted effector T cells but reduces infiltration by regulatory T cells. This resulted in ameliorated cancer control in various models of breast cancer, non-small cell lung cancer and cutaneous fibrosarcoma.

In conclusion, ACBP/DBI can be considered as a gerogene product (or geroprotein) that contributes to the development of cancer as well. This pro-carcinogenic effect of ACBP/DBI may involve its capacity to inhibit autophagy, to stimulate inflammation and to inhibit immunosurveillance.

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EXPANSION UNDER IL-2 REMOVES PRIOR EXHAUSTION AND REPROGRAMS TUMOR-REACTIVE CD8$^+$ TIL INTO NON-CANONICAL EFFECTOR CELLS WHICH DRIVE THERAPEUTIC SUCCESS IN TIL THERAPY

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Adoptive cell therapy (ACT) with autologous tumor-infiltrating lymphocytes (TILs) refers to a highly personalized immunotherapy modality that uses multiple fragments of resected tumors as starting material for *ex-vivo* isolation and expansion of polyclonal tumor-resident T cells, in the presence of recombinant interleukin-2 (IL-2), anti-CD3 antibodies and irradiated feeder cells. The cell product is then infused back to the patient conditioned with lymphodepleting chemotherapy, followed by a short course of intravenous administration of high-dose IL-2. TIL-ACT is presently approved for melanoma and under investigation for a variety of epithelial tumors. An increased frequency of highly functional CD8$^+$ T cells that directly recognize tumor cells, and their differentiation status in the therapeutic product, are key determinants of therapeutic efficacy in TIL-ACT. Indeed, TIL products enriched in tumor-reactive CD8$^+$ T cells with stem-like features are associated with clinical response. These two traits suggest a possible *ex-vivo* functional reinvigoration of tumor-reactive CD8$^+$ TILs.

The majority of tumor-reactive CD8$^+$ TILs in baseline tumors, used for generating therapeutic TIL products, exhibit a TOX$^+$ exhausted/dysfunctional state, which could preclude their mobilization for TIL therapy. Indeed, while precursor exhausted (Pex) CD8$^+$ T cells can be functionally reprogrammed away from canonical exhaustion, terminal exhaustion (Tex) is still regarded as a rather irreversible dysfunctional state, anchored by an irreversible epigenetic architecture. It is thus uncertain whether any therapeutic cells originate directly from the TOX$^+$ terminal exhausted/dysfunctional compartment upon TIL/ACT.

In a clinical study of TIL/ACT in melanoma patients, we have recently reported that tumor-reactive clonotypes, which encompass the exhaustion program in baseline tumors prior to *ex-vivo* expansion, are devoid of the transcriptional and epigenetic marks of exhaustion in the infusion products: In patients who responded to TIL/ACT, we observed that a large fraction of tumor-derived $CD8^+$ clonotypes found in the product were originally in a Pex or Tex state. Moreover, a significantly higher fraction of clonotypes that were found in exhausted states transitioned in products of responding patients relative to non-responders. Moreover, we ideintified 496 $CD8^+$ clonotypes, which could be found exclusively in a single original state in tumors, and tracked them from the tumor to the product. Computing the relative expansion of these clones in products with respect to their original frequency in tumors, we inferred that both Tex and Pex precursors can expand to give rise to the TIL product, with Pex likely displaying higher expansion potential.

Importantly, whereas original tumor-resident $CD8^+$ TILs displayed clear distinct canonical states *in situ*, these exhaustion-specific transcriptional signatures were largely attenuated in the TIL/ACT products. Concurrently, features of effector cells, in particular cytotoxic genes including granzymes (*GZMA*, *GZMB*, and *GZMH*), granulysin (*GNLY*), perforin (*PRF1*), *NKG7*, and *CX3CR1*, were upregulated upon expansion. We tracked a total of 216 individual tumor-reactive $CD8^+$ clones identified in single cell data in baseline tumors, TIL/ACT product, and a tumor biopsy 30 days post-infusion. Strikingly, *in vitro* expanded TIL displayed significantly weaker exhaustion signatures, which was particularly obvious in responding patients, where key exhaustion markers, including the master regulator of T cell exhaustion *TOX*, as well as *PDCD1*, *HAVCR2*/*TIM3*, *TIGIT*, *LAG3*, and *CXCL13*, were markedly downregulated relative to the same clones in baseline tumors. Regulon analyses also suggested that clonotypes transitioned away from exhaustion. Importantly, cytotoxic molecules such as *GNLY* and *GZMB* were upregulated along with *CXCR3*, a marker associated with tumor infiltration.

When we interrogated the the tumor-reactive clonotypes in day-30 biopsies of responding patients, they were found to have preserved post-transfer the new transcriptional states acquired during expansion, including proliferation and effector features, while they had acquired an attenuated state of exhaustion. Interestingly, *TOX*, which was lost upon expansion, was re-upregulated in tumors of responders by day 30, albeit at lower levels than baseline tumors. Moreover, the cells expressed high levels of *TNFRSF9*, *PRF1*, *IFNG*, *CD69*, *GNLY* and *GZMA*. These results suggested that TIL expansion drove important cell reprogramming, resulting in functionally reinvigorated effector cells which are also able to retain activated effector-cell features and are capable of driving objective tumor responses in

patients who failed prior PD-1 therapy.

Although previous studies have shown that the terminal exhaustion may be partially reversed in Tpex cells, once reached, terminal exhaustion is regarded as a rather irreversible dysfunctional state, anchored on an irreversible epigenetic architecture. Presently, there is no direct evidence that individual terminal exhausted $CD8^+$ TIL clones can be reprogrammed. To address this key question, we used bone-marrow chimeric mice to effectively track monoclonal tumor-reactive $CD8^+$ TILs throughout the *ex-vivo* TIL manufacturing process. Strikingly, we found that *ex vivo* manufacturing protocols with high-dose IL-2 "erase" most of the transcriptional and epigenetic commitments in tumor-reactive terminal exhausted $CD8^+$ TILs, previously acquired during their residency within the tumor microenvironment. Notably, this process repositions former precursor and terminal exhausted $CD8^+$ TILs away from the evolutionary constrained space of transcriptional and epigenetic cell states, to acquire fully functional, superior non-canonical effector cell states, where less differentiated canonical $CD8^+$ TIL subsets also converge during expansion.

Thus, *in vitro* high-dose IL-2 supplementation acts as a cell state dominant factor, that forces the acquisition of similar final non-canonical effector states regardless of the initial phenotype. Altogether, our observations could explain why functionally reinvigorated tumor-reactive TIL-ACT products can drive impressive tumor regression in advanced clinical settings and pave the way for reimagining the potential synthetic evolutionary space of T cell states beyond what natural evolution has yielded, to produce superior synthetic cells capable of eradicating advanced solid tumors.

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LANGUAGE MODELS TO ACCELERATE NEOANTIGEN-SPECIFIC TCR DISCOVERY FOR CANCER IMMUNOTHERAPY

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Tumor evolution is shaped by a delicate balance: while mutations may provide proliferative advantages, they can also generate novel peptides that render tumor cells visible to the immune system (*1*, *2*). The ability of T cells to detect such neoantigens is central to immune surveillance and has inspired therapeutic strategies such as adoptive transfer of TCR-engineered T cells (TCR-T) (*3*). However, application of this strategy has been limited by a critical barrier—the difficulty of identifying, from billions of possible clonotypes, the rare TCRs that recognize tumor-specific mutations with functional activity. Current discovery pipelines are slow, resource-intensive, and often impractical for patients with aggressive disease.

This challenge arises from both biological and technical complexity. Each tumor has a distinct mutational landscape, and only a small fraction of alterations yield processed and presented peptides that can bind TCRs with sufficient affinity. Even within tumors, most infiltrating lymphocytes are not tumor-specific but represent bystander responses. Thus, the bottleneck in TCR-T development is not simply screening capacity, but the ability to efficiently identify the few clonotypes that mediate meaningful immune recognition (*4*).

Checkpoint blockade therapies have revolutionized outcomes in melanoma and other cancers (*5*), but their efficacy depends on the presence of a preexisting repertoire of tumor-reactive T cells. Many common tumors, especially those with low mutational burden, lack such repertoires and remain refractory to checkpoint inhibition. TCR-T therapy offers a complementary path by introducing new, personalized repertoires of antitumor lymphocytes. The central question becomes how to accelerate receptor discovery to make such therapies feasible at scale and across cancer types.

Recent progress in single-cell sequencing has opened new opportunities. By simultaneously capturing transcriptomes and paired αβTCR sequences from thousands of tumor- infiltrating lymphocytes (TILs), we can now reconstruct the cellular states that distinguish reactive from bystander populations. Across cancers, tumor-reactive T cells consistently display distinctive transcriptional profiles characterized by effector activity, cytotoxicity, and features of chronic stimulation (*6*). These programs serve as natural barcodes of reactivity, providing a way to enrich for clonotypes most likely to recognize tumor-derived peptides.

Yet transcriptional context alone cannot reveal neoantigen specificity. At the molecular level, recognition depends on the structural and biochemical properties of the TCR–peptide–HLA interface (*7*). Subtle changes in complementarity-determining region loops or residue chemistry can alter binding dramatically (Figure 1). Traditional alignment-based methods often fail here: clonotypes with divergent sequences may converge on the same antigen, while nearly identical sequences may diverge in specificity. This complexity has motivated new computational approaches inspired by advanced language models.

We developed a protein language-based framework that integrates primary amino acid sequence with predicted three-dimensional features of αβTCRs. Trained on more than 800,000 paired receptors linked to over 3,000 neoantigens, the model learns the molecular grammar and semantics of immune recognition. The embeddings it generates cluster receptors according to shared specificity even without peptide information, demonstrating that they capture convergent biophysical constraints.

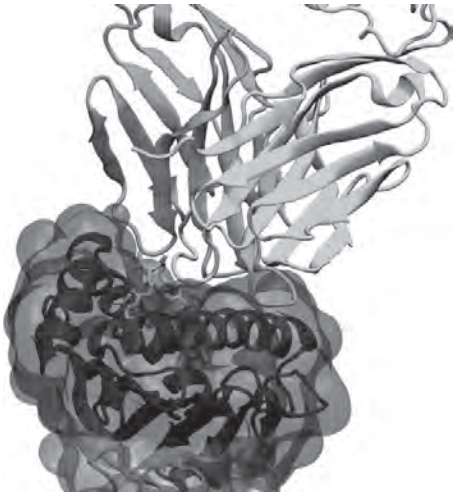

Figure 1 Structural model of a TCR–neopeptide–MHC complex. TCR α- and β-chains (orange, yellow) dock diagonally over a peptide–MHC class I complex (blue), contacting both peptide (green) and MHC helices.

By integrating single-cell profiling of T cell states with structural receptor information, we established a multimodal discovery pipeline. Transcriptomic profiles point to the clonotypes most enriched for tumor recognition, while structural modeling refines specificity predictions. Combined, these modalities reduce the search space dramatically and allow candidate TCRs to be prioritized for functional testing. This framework turns a once intractable search into a tractable, accelerated process.

Our analyses demonstrate the potential of this framework. In microsatellite-stable colorectal cancer—a disease resistant primarily to checkpoint blockade—more than a dozen functional receptors were discovered from a single patient sample within weeks. These included clonotypes recognizing mutations in oncogenes such as *KRAS* and *TP53*. Similar applications in other tumor types have yielded dozens of validated TCRs, far exceeding the efficiency of conventional pipelines. Comparative analyses of matched primary and metastatic lesions show that tumor-reactive states can be both conserved and remodeled, reflecting how immunity itself evolves under selective pressure.

These advances establish an iterative computational–experimental loop. Predictions nominate candidate receptors; cloning and functional assays validate activity; results are fed back into the model to improve subsequent predictions. This cycle reduces discovery timelines from months to weeks, enabling integration into ongoing clinical trials. Beyond therapeutic application, curated libraries of validated receptors generated through this process illuminate fundamental rules of immune recognition and provide a foundation for future therapies.

The implications extend broadly. Scientifically, this framework decodes the molecular grammar of TCR recognition, linking immune cell states with the structural determinants of specificity. Translationally, it provides a scalable platform for TCR-based therapies, addressing the bottleneck that has limited progress in the field. Just as recent predictive models have shown that checkpoint blockade outcomes can be predicted (*8*), our approach demonstrates that artificial intelligence applied at the mechanistic level of TCR recognition can accelerate the discovery of therapeutic receptors. In my presentation, I will describe the development of this framework, highlight validation results, and discuss its integration into future immunotherapy trials.

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NEW FUNCTIONAL GENOMICS TOOLS TO ELUCIDATE CANCER-SPECIFIC RNAS AND THEIR REGULATION

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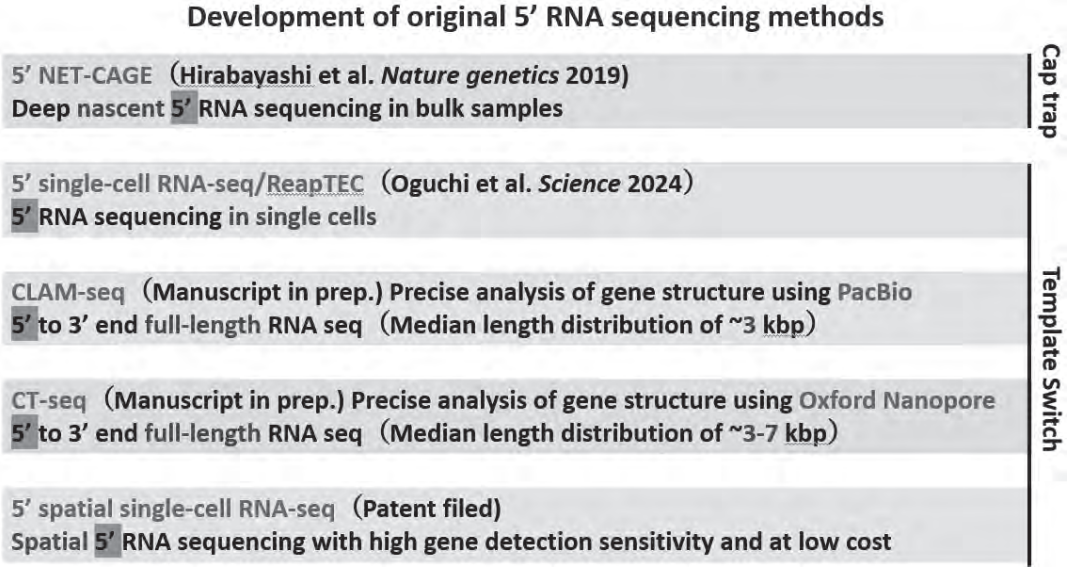
We are developing original transcriptomic technologies to investigate previously uncharacterized aspects of the cancer transcriptome and to elucidate its interactions with the tumor microenvironment, including immune and stromal cells (**Figure**).



The transcriptional landscape of cancer cells is orchestrated by the spatiotemporal activity of cis-regulatory elements, such as promoters and enhancers. To decode the regulatory logic underlying cancer-specific gene expression, we have developed a suite of innovative

transcriptomic technologies. These include native elongating transcript-cap analysis of gene expression (NET-CAGE), which sensitively captures transcription start sites of nascent RNAs—including unstable enhancer RNAs (Hirabayashi et al. *Nature Genetics* 2019)—and a novel 5′ single-cell RNA sequencing (5′ scRNA-seq) approach that enables the identification of functional enhancers across heterogeneous cell populations (Oguchi et al. *Science* 2024).

To overcome the limitations of conventional short-read RNA sequencing, we have also developed full-length RNA sequencing methods that reconstruct complete RNA molecules, including unannotated long non-coding RNAs and novel protein-coding gene candidates. Applied to hundreds of cancer and normal tissue samples, these technologies have uncovered thousands of previously unknown transcripts with potential roles in tumorigenesis. More recently, we have been advancing single-cell spatial transcriptomic methods that integrate long-read sequencing with spatial resolution, allowing us to map full-length RNAs—including novel cancer-specific human genes—within the tumor microenvironment. This enables high-resolution, in situ analysis of cancer cells, immune populations, and stromal compartments, as well as their spatial dynamics and evolutionary trajectories.

By integrating these next-generation transcriptomic tools with clinical data, we aim to systematically chart cell-type–specific regulatory elements and cancer-specific RNAs, including novel transcripts uniquely expressed in human tumors. Our ultimate goal is to translate these insights into novel therapeutic targets and predictive biomarkers. In this talk, I will present our latest methodological innovations and findings, and discuss their implications for understanding cancer biology, tumor evolution, and the development of precision cancer therapies.

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GENOMIC, HOST, AND MICROENVIRONMENTAL DETERMINANTS OF CANCER PROGRESSION

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Cancer is a disease of the genome, driven by somatic mutation and evolutionary selection, and continually shaped by immune recognition and elimination. My research has repeatedly shown that tumor-intrinsic features predict progression and outcome across diverse cancers, enabling disease forecasting while defining new targets [1-7]. For example, we described a 'big bang' model of tumor growth wherein malignant and even metastatic potential is specified early such that some tumors are 'born to be bad' [1]. Similarly, we demonstrated that breast cancer recurrence risk could be predicted decades prior based on somatic genomic copy number aberrations [5-7].

These findings sparked other questions. Do hereditary or host differences influence somatic evolution and the molecular subtype of disease an individual develops? *When and how do* molecular differences in tumors of the same histology arise? For example, germline BRCA1 mutations are associated with a 55-72% lifetime risk of breast cancer, enriched for estrogen-receptor negative (ER-) disease, while BRCA2 mutations are associated with ER+ disease. However, the basis for these molecular differences is poorly understood. More generally, sporadic cancers are assumed to result from random mutations acquired during cell division and hence attributable to 'bad luck'. Our findings suggest that this process is not random but influenced by one's germline genome and immune system – and hence can be predicted.

Analyzing 6,000 patients with pre-invasive, invasive or metastatic breast cancer using a powerful computational framework that accounts for ancestry, germline genotype, and HLA (human leukocyte antigen), we discovered a pivotol role for hereditary and immune features in dictating the subtype of BC and its aggressiveness [8]. Specifically, hereditary variants in

recurrently amplified oncogenes, such as HER2, combined with a patient's HLA, dictates the levels of oncogene-derived epitopes that can be presented for recognition by the host's immune system. Thus, when specific combinations of HLA haplotype and seemingly innocous genetic variants in these oncogenes co-occur in the same individual, the immune system's ability to detect and surveill nascent pre-invasive tumor cells that display these epitopes, is corrospondingly impacted. As such, individuals with high germline epitope burden (GEB) in the HER2 oncogene are less likely to develop HER2+ BC compared to other subtypes. Our work indicates that high GEB leads to increased MHC-class I presentation of HER2 epitopes, and that *immunoediting selects against tumor clones* with many copies of HER2.

The same pattern holds true for other subgroups of breast cancer, including the aggressive ER+ luminal tumors, we previously identified via an Integrative Classification scheme (ICs), which have a persistent recurrence risk for two decades [5,6]. These four 'high-risk' ER+ IC subgroups are defined by copy number amplifications, similar to HER2 (IC1: *RPS6KB1*/*PRR11*, IC2: *CCND1*, IC6: *FGFR1*, IC9: *MYC*). Together, these subgroups account for 25% of ER+ disease and most recurrences. These genome unstable subgroups are immune-depleted and refractory to standard-of-care therapies, underscoring the need for need for new approaches. Moreover, the high-risk ICs are enriched amongst young women. Further, pre-invasive ductal carcinoma in situ (DCIS) are addicted to these same oncogenes [7].

While high GEB is protective in DCIS owing to immune-surveillance and elimination, it is a poor prognostic factor in invasive breast cancer and accompanied by a "switch" to immune tolerance. Indeed, low GEB is associated with progression to invasive breast cancer. Invasive tumors that overcome this immune-mediated negative selection are immune cold and myeloid enriched with greater propensity to recur, emphasizing the importance of earlier interventions. I will discuss ongoing efforts to elucidate the molecular basis for immune suppression in these tumors through multi-modal spatial profiling of a large collection of breast tumors sampled throughout disease progression.

Further, I will review new data demonstrating that the patterns of germline-mediated immunoediting we first described in breast cancer, extends to diverse solid tumors, providing a redout of pre-existing immunity to tumor-associated antigens. Our findings also unearth a previously unappreciated source of clonal antigens that may be exploited to treat and intercept aggressive tumors. Finally, I will discuss how this provocative discovery helps to explain the *missing heritability* of malignancy – with implications for risk stratification and predicting cancer risk in healthy individuals.

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ADVANCING T CELL THERAPIES FOR SOLID TUMORS THROUGH TARGET DISCOVERY, METABOLIC MODULATION, AND EXHAUSTION RESISTANCE

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Despite the transformative impact of immune checkpoint blockade (ICB) in advanced melanoma, a significant proportion of patients do not derive clinical benefit or eventually develop resistance, underscoring the need for effective immunotherapies for ICB-refractory disease. T cell-based strategies are a promising alternative. We are pursuing this approach for advanced melanoma and other solid tumors along two main lines: developing Chimeric Antigen Receptor (CAR) T cell therapies and advancing tumor-infiltrating lymphocyte (TIL) therapy.

We have developed a novel CAR T cell targeting TYRP1, a melanoma differentiation antigen expressed across cutaneous, acral, and uveal melanomas, which is barely expressed on healthy tissues, making it a safe target. TYRP1-targeted CAR T cells exhibit antigen-specific, MHC- independent against human cutaneous and acral melanoma cell lines in vitro (**Figure 1**). More importantly, in human melanoma xenograft models, they slow tumor growth without causing the pigmentation-related toxicities observed with TCR-based approaches (1). These findings support TYRP1-CAR T cells as a promising therapeutic platform for melanoma.

In parallel, we are advancing CAR T cell therapy for bladder cancer (BCa), where current intravesical treatments are limited by toxicity and recurrence. Using a transcriptomics-guided pipeline, we identified MUC16 as a clinically relevant target enriched in aggressive BCa subtypes. We engineered a mesothelin (MSLN)-based CAR that selectively targets MUC16+ BCa cells. Intravesical delivery of MSLN-based CAR T cells in xenograft models

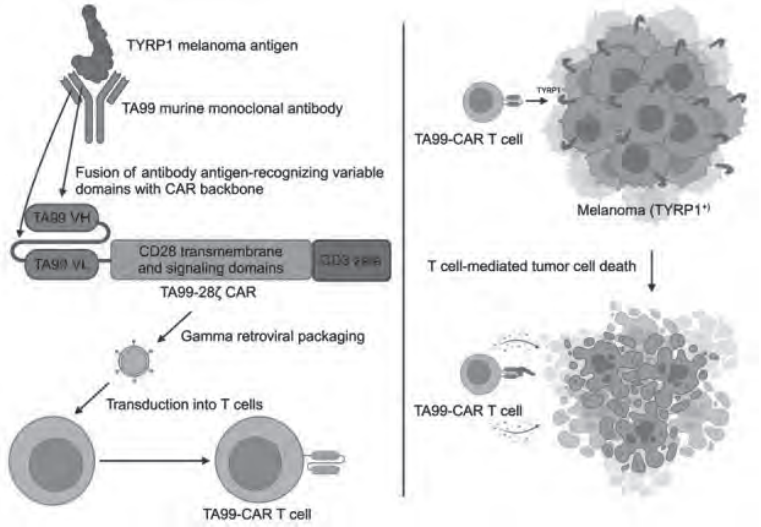

Figure 1 Scheme for TYRP1-targeted CAR T cell generation (left). TYRP1-targeted CAR T cells kill human melanoma expressing TYRP1 (right).

resulted in superior tumor control and localized immune activation, with minimal systemic toxicity. These results support the development of locoregional CAR T cell therapies for organ-confined solid tumors.

Beyond target discovery, we are investigating how the tumor metabolic state shapes T cell function. In melanoma and triple-negative breast cancer (TNBC), we showed that inhibiting lactate dehydrogenase (LDH), a critical glycolytic enzyme, either genetically or pharmacologically enhances ICB efficacy by increasing glucose availability for effector T cells and destabilizing regulatory T cells (2, 3) (**Figure 2**). Both human and mouse tumors exhibit higher LDH expression, glycolytic rates, and glucose uptake than normal tissues and activated T cells, making LDH a rational target to preferentially disrupt tumor glycolysis without impairing T cells. We are now leveraging these metabolic interventions to remodel the microenvironment of highly glycolytic tumors, aiming to enhance adoptive cell therapies, particularly given the recent FDA approval of TIL therapy for advanced melanoma and the growing interest in CAR T cell therapies for solid tumors.

We have also identified a novel mechanism of T cell exhaustion mediated by the extracellular matrix protein thrombospondin-1 (TSP-1) and its receptor CD47. TSP-1-CD47 engagement on CD8+ T cells activates the calcineurin-NFAT-TOX axis, promoting exhaustion and impairing effector function (**Figure 3**). Blocking this interaction with a selective peptide prevented T cell exhaustion and controlled tumor growth in melanoma and colon carcinoma

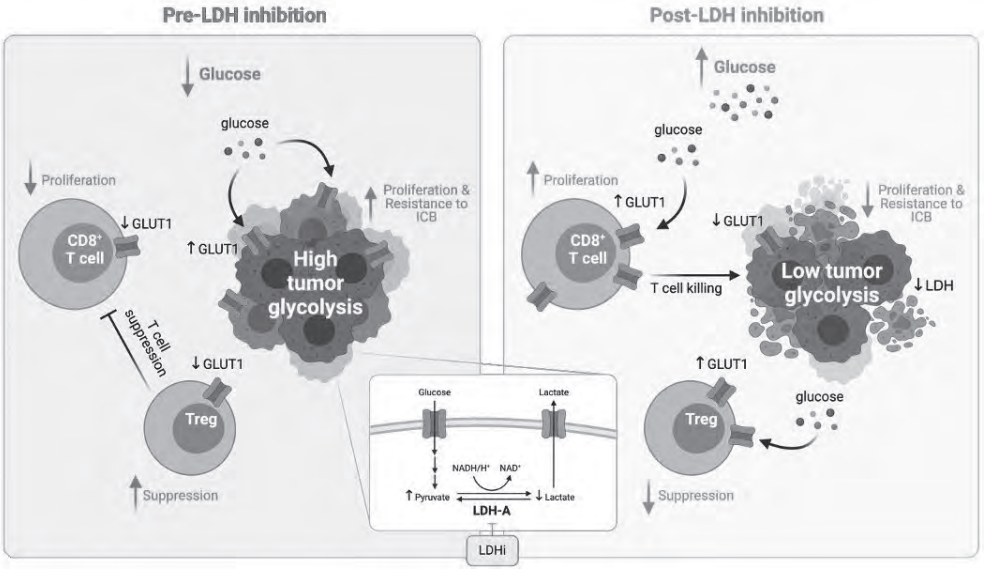

Figure 2 Targeting tumor glycolysis by inhibiting LDH restore effector T cell function by increasing glucose availability in the extracellular environment and destabilizing Tregs.

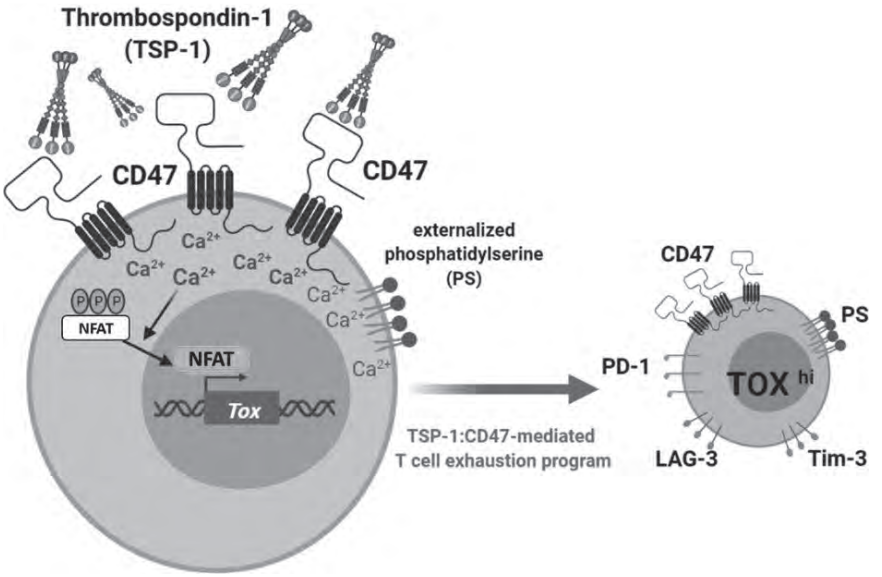

Figure 3 Proposed model illustrating that engagement of the extracellular matrix protein TSP-1 to CD47 on the surface of CD8+ T cells drives their progression toward T cell exhaustion.

models. Targeting the TSP-1-CD47 axis may therefore offer a new strategy to prevent or reverse exhaustion, enhancing the durability of T cell-based therapies.

Together, these efforts reflect our commitment to translating mechanistic insights into therapeutic advances, with the goal of overcoming resistance and improving outcomes for patients with solid tumors. My discussion will focus on the development and optimization of adoptive cell therapies.

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DRIVING NEW CAR-T CELLS

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Dr. Maus directs a comprehensive bench-to-bedside and back program in cellular immunotherapy. The program is composed of a "research and discovery" arm that designs and examines the novel CAR-T cells, "a regulatory/translational" arm to test the CAR-T cells in human subjects, and a "reverse translation" arm to learn how the CAR-T cells engraft, persist, and function following infusion into patients. From this knowledge, we then design and evaluate the next generation of CAR-T cells that are even more likely to eliminate their target tumor. Using this approach, we are generating a pipeline of genetically engineered CAR-T cells to use as "living drugs" in patients with cancer and autoimmune diseases.

1. Identify novel target antigens and engineering strategies to improve CAR-T cell recognition of target cells

We are developing antigen receptors and secreted molecules to target new tumor antigens and/or multiple antigens at a time on tumor cells with the aim of improving the elimination of heterogenous tumor cells and preventing antigen-negative relapse while decreasing the risk of targeting healthy cells. We have recently published on our experience in targeting wild-type EGFR in glioblastoma using a novel technology whereby CAR-T cells secrete T cell engagers, and this has opened a therapeutic window to maximize anti-tumor effects and minimize on-target off-tumor toxicity. This idea was conceived in the laboratory, tested preclinically in newly designed murine xenograft models, and then translated to a first-in-human clinical trial. Rapid and dramatic regressions of glioblastoma were observed in the first patients, and interventions to improve the durability of the effects are now underway

and clinical data will be updated.

2. Combine CAR-T cells with other drugs to sensitize tumors to T cell-mediated killing, potentiate T cell function, or improve safety.

Many of the small molecule drugs and antibodies used in the clinic exert their effects on signaling pathways in tumor cells, T cells, and other immune cells. We aim to discover synergistic drug/T cell combinations to increase safety and efficacy, and use genetic engineering tools to confer specific drug sensitivity, resistance, or enhanced molecular switches. We have had significant focus on the role of cell death and apoptosis in particular to drive an understanding of how CAR T cells exert anti-tumor effects and what underlying mechanisms can make tumors resistant to CAR T cells.

3. Use in vivo screens to rapidly identify CAR-T cell designs that improve efficacy

We have predicted several modifications to CAR-T cells that could improve their efficacy, but testing them one at a time is very time consuming. To speed up this process, we have developed an in vivo screening technique where a pool of CAR T cells with different modifications is injected into mice with tumors and the one(s) that best infiltrate the tumor and expand are identified over time. Doing this in vivo increases our chances of identifying designs that will work in patients.

4. Understand how CAR-T cells are functioning in patients.

After translating our novel CAR-T cells from the lab to the clinic, we carefully follow how CAR-T cells expand, persist, and/or change transcriptional and traditional phenotype over time in patients.

5. Dissect cytokine pathways and their role in CAR-T cell activities, including anti-tumor effects and toxicities.

We use hypothesis-based testing, pharmacologic blockade, and genetic approaches in various humanized, murine, and ex vivo models to dissect the roles of various cytokines, cytokine receptors, and cell-cell interactions which may play a role in the clinical manifestations of CAR T cell therapies.

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UNDERSTANDING THE MOLECULAR MECHANISMS OF CAR-T CELL THERAPY FAILURE

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The adoptive transfer of chimeric antigen receptor (CAR)-engineered T cells has become the standard of care for patients with relapsed and refractory B-cell malignancies and multiple myeloma. However, a substantial proportion of patients treated with CAR-T cells eventually relapse, even after achieving complete remission.

Therapeutic failure is at least partly attributable to the dysfunction of infused CAR-T cells, including terminal differentiation and exhaustion induced by repeated antigen encounters. Notably, CAR-T cells undergo distinct epigenetic alterations, including DNA methylation and histone modifications, leading to irreversible impairment of their effector functions. Modulating key regulatory factors can suppress or even reverse these dysfunctional processes.

Our previous efforts focused on modulating CAR-T cells during ex vivo expansion, a process in which precocious T-cell differentiation accompanies massive proliferation. We found that pharmacological inhibition of BET bromodomain proteins significantly maintained an early memory phenotype in cultured T cells [1]. BET inhibitor–treated CAR-T cells exhibited superior persistence and durable antitumor efficacy in multiple mouse tumor models.

Although this strategy successfully generates higher-quality antitumor T cells, these cells rapidly differentiate *in vivo* upon antigen encounter. Stable modification of T-cell properties at the genetic level may result in more durable efficacy. Recent advances in genome-editing technologies have enabled efficient ablation of specific genes in primary T cells. We recently demonstrated that genetic ablation of *PRDM1* promotes the persistence of CAR-T cells with a memory-like phenotype [2]. PRDM1 ablation induced global transcriptional

reprogramming accompanied by concordant epigenetic changes in peripheral blood T cells (Figure 1).

A remaining limitation of this modification is that *PRDM1* knockout simultaneously compromises effector function and increases susceptibility to exhaustion. Our current study focuses on introducing additional modifications to *PRDM1*-knockout CAR-T cells to restore their effector function upon repeated antigen stimulation.

Another key strategy to enhance CAR-T cell efficacy is the provision of cytokine signaling. We previously incorporated a cytokine signaling domain into the intracellular portion of the CAR (JAK–STAT CAR) [3]. Anti-CD19 JAK–STAT CAR-T cells exhibited superior antitumor efficacy in preclinical models and are currently being evaluated in clinical trials. While cytokine signaling can improve therapeutic outcomes, excessive activation is also associated with safety concerns such as cytokine release syndrome (CRS) and neurotoxicity. Clinical data indicate that robust expansion of infused CAR-T cells correlates with the development of high-grade CRS.

To simultaneously enhance both efficacy and safety, we recently developed the chimeric cytokine receptor G6/7R, which scavenges IL-6 and delivers constitutive IL-7 signaling [4, 5]. G6/7R CAR-T cells exhibited superior proliferative capacity while suppressing excessive serum IL-6 elevation in humanized mouse models. Using this platform, we are now preparing for clinical trials targeting patients with lymphoma.

In addition to functional impairment of CAR-T cells, tumor cells may possess or acquire intrinsic mechanisms to evade T cell–mediated attack through genetic and metabolic alterations. We have recently identified multiple molecular mechanisms exploited by cancer cells to acquire resistance to T cell–mediated cytotoxicity through pan-cancer screening. Targeting these resistance mechanisms can render tumor cells susceptible to a variety of immunotherapeutic strategies.

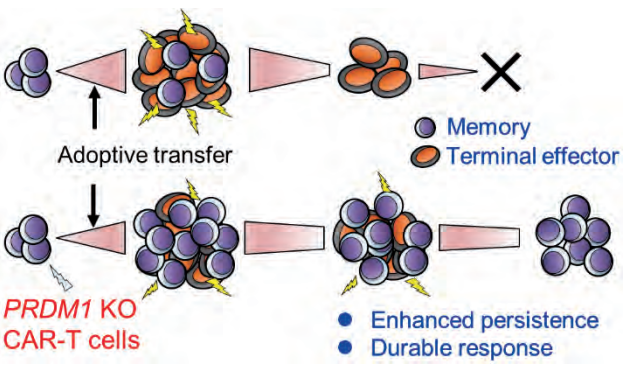

Figure 1 CAR-T cells lacking *PRDM1* exhibit early memory phenotypes and enhanced longevity.

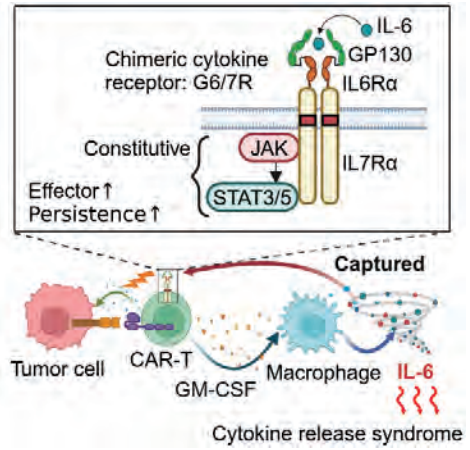

Figure 2 Development of a chimeric cytokine receptor to enhance the efficacy and safety of CAR-T cells. G6/7R-expressing CAR-T cells sequester extracellular IL-6 and exhibit long-term proliferative capacity via constitutive IL-7 signaling.

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BUILDING ON CD19 CAR THERAPY TO TACKLE SOLID TUMORS

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The genetic engineering of T cells offers a means to repurpose immune cells to remedy the limitations of natural immune responses. The first successful embodiment of engineered immunity is chimeric antigen receptor (CAR) therapy targeting CD19. CARs that target CD19, a cell surface molecule found in most leukemias and lymphomas, have produced remarkable responses in patients with refractory B cell malignancies. Five CD19 CAR therapies are currently approved by the US FDA. Over 1500 CAR trials are listed on the clinicaltrials. gov website, with CD19 remaining the most frequent target across both oncology and autoimmunity. Despite high complete remission rates obtained following a single CAR T cell infusion in patients with relapsed hematological malignancies, many patients will eventually relapse, pointing to the need to further improve CAR design and T cell engineering strategies. CAR T cells have not yet succeeded to overtake solid tumors, further pointing to the need to further adapt CAR T cell design.

Several gaps in the activity of current CAR T cells are well recognized: insufficient antigen sensitivity, insufficient functional persistence, inability to enter cold tumors, and inability to overcome strongly immunosuppressive or fibrotic tumor microenvironments. Target identification poses additional challenges that stem from tumor heterogeneity and target expression in normal cells (the latter resulting in on-target toxicities). Recent studies on the antigen sensitivity of different CAR designs have yielded valuable insights into the minimum antigen density required for effective tumor engagement by CAR T cells. A novel family of CARs, termed HIT receptors, provides greater sensitivity and enables elimination of tumors that escape conventional CARs. Logic-gated CAR T cells offer the prospect of more selective tumor targeting, as exemplified by IF-BETTER gating to direct context-

dependent antigen sensitivity. Novel CAR designs, such as 1XX and 1A CARs, which respectively calibrate strength of activation and mRNA translation, combine the effector potency of CD28-based CARs with the T cell persistence afforded by 4-1BB-based CARs. Genome editing is emerging as a valuable tool to transcriptionally control CAR expression and enable in vivo screens that reveal novel transcriptional regulators of the functional persistence of CAR T cells. Piece by piece, these advances are setting the stage for successful attempts to adapt the CAR technology to solid tumors.

CAR T cells embody a novel paradigm for immunotherapy, providing "living drugs" to patients who fail to on their own generate effective tumor immunity through active immunization or checkpoint blockade. The successes and limitations of CD19 CAR T cells provide a roadmap for future CAR therapies to tackle solid tumors.

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EMERGING CONCEPTS IN CAR T CELLS

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Chimeric antigen receptor expressing T cells have transformed the therapeutic landscape for high-risk B cell and plasma cell malignancies and early signs are activity are emerging against solid tumors and autoimmune disease. Immense progress in synthetic biology, cell manufacturing and T cell engineering is fueling an innovation pipeline in this arena that is well positioned to sustain advances for patients via this novel class of therapeutics. This presentation will share insights gleaned from studies conducted in the Stanford Center for Cancer Cell Therapy, emphasizing patient centered discovery research to inform mechanisms of resistance and creation of next generation therapeutics designed to overcome resistance. Areas of focus will include promising activity of GD2-CARs in diffuse midline gliomas, emerging understanding regarding mechanisms of resistance to CAR T cell therapies and new synthetic biology platforms designed to enhance potency and diminish toxicity.

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TARGETING THE TUMOR MICROENVIRONMENT FOR EFFECTIVE PROSTATE CANCER THERAPY

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1. Introduction

Cellular senescence is a stable cell cycle arrest occurring in diploid cells that limits their proliferative lifespan. Senescent cells accumulate with age at sites of age-related pathologies[1], and can impair tissue physiology, leading to progressive functional decline. In cancer, therapy-induced senescent cells contribute to tumor progression and treatment resistance. Here, I outline my laboratory's contributions to advancing the understanding of senescent tumor cell biology and its interaction with the tumor immune response.

2. The interplay between senescence and myeloid inflammation

The interplay between senescent tumor cells and immune cells is dynamic and profoundly influences tumor progression and therapeutic responses. Although senescent tumor cells have exited the cell cycle, they remain metabolically active and exert profound effects on the tumor microenvironment (TME) through the senescence-associated secretory phenotype (SASP). In early tumor lesions, the SASP can promote immune surveillance by recruiting macrophages, NK cells, and T cells to the tumor site[2]. These immune cells can recognize and eliminate senescent tumor cells in a process known as senescence surveillance, thereby limiting tumor progression. In advanced disease, however, this interaction becomes more complex. In late-stage tumors, the SASP can shift toward a pro-tumorigenic profile, attracting immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs). These cells can inhibit effector T and NK cells, fostering an environment that supports tumor proliferation, immune evasion, and resistance to therapy[3-7]. In this context, my team has contributed to key advances in myeloid cell biology by demonstrating that these cells can

regulate senescence within tumors, suppress anti-tumor immunity, and promote tumor progression through multiple mechanisms.

3. Impact of myeloid cells on cellular senescence in cancer

We demonstrated that myeloid cells could interfere with the senescence process within the prostate TME[6]. We showed that MDSCs can suppress therapy-induced senescence triggered by chemotherapy or radiation, allowing tumor cells to bypass this protective growth arrest and resume proliferation. This senescence escape not only sustains tumor growth but also creates conditions that favor further genetic instability, leading to more aggressive and treatment-resistant cancer phenotypes[6]. Mechanistically, we identified interleukin-1 receptor antagonist (IL-1RA) secretion by MDSCs as a key mediator, as IL-1RA inhibits the SASP[6]. These findings suggest that disrupting the interaction between senescent tumor cells and MDSCs - such as through pharmacological CXCR2 inhibition - may reinforce therapy-induced senescence and restore adaptive immune function in prostate cancer. Notably, this mechanism is likely relevant beyond prostate cancer, as MDSC-mediated immune suppression via CXCR2 has been described across multiple tumor types.

In parallel, we discovered that tumor-associated macrophages (TAMs) can be reprogrammed to induce tumor cell senescence[5]. CXCR2 inhibition repolarizes TAMs from a tumor-promoting to an anti-tumorigenic phenotype[5]. Re-educated macrophages begin to secrete tumor necrosis factor-alpha (TNFα), a cytokine with potent pro-inflammatory and anti-tumor properties[5]. TNFα-driven senescence contributes to the suppression of tumor growth and the stabilization of the cancerous state, preventing further progression.

Collectively, these studies highlight CXCR2 inhibition in myeloid cells as a dual therapeutic strategy in advanced prostate cancer, simultaneously preventing senescence escape mediated by MDSCs and promoting senescence induction through reprogrammed TAMs.

4. Targeting senescence in the immune system

Senescence in immune cells can impact various aspects of the immune response, contributing to reduced immunity and increased susceptibility to infections, cancer, and chronic diseases, particularly in the elderly. Most studies of immune senescence have focused on T cells, whose exhaustion and senescence have been reported across multiple cancers[8], while the ability of myeloid cells to undergo senescence has remained largely unexplored[7]. Recently, our team demonstrated that polymorphonuclear-MDSC (PMN-MDSCs) can undergo cellular senescence while remaining functionally active within the prostate cancer TME[3]. Tumor-derived apolipoprotein E (ApoE) can induce a senescent-like state in PMN-MDSCs by binding to TREM2[3]. These senescent-like PMN-MDSCs persist in

the TME and sustain chronic inflammation and immunosuppression that promote tumor growth and resistance to therapy[3]. These cells secrete a specific set of cytokines and pro-inflammatory factors, contributing to a tumor-promoting environment and limiting the efficacy of immunotherapy by suppressing T cell activity.

Importantly, we showed that therapeutic elimination of senescent PMN-MDSCs enhances the efficacy of enzalutamide in multiple mouse models. A screening of 500 compounds identified an HDAC inhibitor as a potent immunosenolytic agent capable of selectively depleting senescent-like myeloid cells from the TME, effectively reducing their tumor-promoting effects[3].

5. Role of inflammatory myeloid cells beyond senescence

One of our key discoveries was that PMN-MDSCs act as critical paracrine mediators of prostate cancer progression, especially in castration-resistant prostate cancer (CRPC)[4]. In both CRPC mouse models and patient samples, we showed that tumor-infiltrating myeloid cells express high levels of the inflammatory cytokine IL-23. Elevated IL-23 expression was associated with resistance to androgen-deprivation therapy (ADT)[4]. Accordingly, we provided evidence that IL-23 blockade can prevent or overcome resistance in CRPC[4]. We further identified the chemokine receptor CXCR2 as a key regulator of PMN-MDSC recruitment to the TME, and showed that its inhibition suppresses PMN-MDSC infiltration, and enhances ADT efficacy in prostate cancer[3-5]. In a follow-up study, we characterized the PMN-MDSC secretome and identified these cells as a major extrahepatic source of coagulation Factor X (FX) in the TME[9]. PMN-MDSC-derived FX promotes androgen-independent tumor growth via paracrine activation of protease-activated receptor 2 (PAR2) on prostate tumor cells, contributing to therapy resistance across several mouse models, including those treated with anti-IL-23 antibodies and CXCR2 inhibitors[6]. Notably, we provided preclinical evidence that direct FXa inhibitors can counteract therapy resistance and significantly enhance the efficacy of enzalutamide in prostate cancer[9].

6. Clinical translation of the findings

Our discoveries on myeloid cell biology and senescence in prostate cancer have inspired the design of a clinical trial evaluating the MDSC inhibitor AZD5069 in combination with androgen receptor signaling inhibitors in metastatic CRPC (mCRPC) patients. We conducted an international phase 1, multi-centre, single-arm, open-label trial (ClinicalTrials.gov identifier: NCT03177187) across three European centers, enrolling mCRPC patients progressing after at least one androgen-receptor inhibitor[10].

The combination of CXCR2 inhibition with androgen receptor blockade (e.g., enzalutamide) was well tolerated and provided durable clinical benefit, including

biochemical and radiological responses in a subset of patients. Translational analyses revealed that neutrophilia correlated with tumor cell senescence-associated transcriptional programs, while elevated CXCR2 ligand levels were associated with increased myeloid infiltration and poorer prognosis. Consistently, inhibition of myeloid cell infiltration reduced the immunosuppressive and pro-tumorigenic signaling within the TME, restoring therapeutic efficacy[10]. Moreover, the combination therapy also enhanced cytotoxic T cell infiltration and activity.

These findings support the clinical development of CXCR2 inhibitors as adjunct therapies in prostate cancer treatment, particularly for patients with advanced or therapy-resistant forms of the disease and suggest broader applicability in other cancers where myeloid cells play a role in therapy resistance.

Importantly, translational data also revealed upregulation of CXCL1, CXCL2, and CXCL8 following CXCR2 blockade, and co-expression of CXCR1 and CXCR2 on intratumoral MDSCs, indicating that redundant CXCR1 signaling may limit the efficacy of selective CXCR2 inhibition[10]. Based on these insights, we are initiating a new proof-of-mechanism and proof-of-concept clinical trial evaluating dual CXCR1/2 blockade in combination with apalutamide in men with mCRPC.

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TUMOR-PROMOTING SECRETOME FROM SENESCENT CAFS IN THE STEATOTIC LIVER TUMOR MICROENVIRONMENT

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Metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) have emerged as major contributors to hepatocellular carcinoma (HCC). However, the precise mechanisms underlying liver cancer development in these conditions remain unclear. We previously revealed that obesity-induced deoxycholic acid (DCA), a gut microbial metabolite, triggers a senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSCs), a secretome that senescent cells secrete a variety of inflammatory cytokines, chemokines, proteases, and so on. (1)

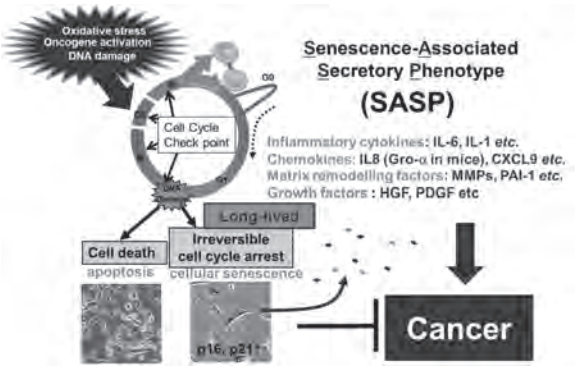

Figure 1 A scheme of senescence-associated secretory phenotype. Cellular senescence functions as an important tumor-suppressor mechanism. However, long-lived senescent cells secrete a variety of secreted proteins, which is a phenotype called senescence-associated secretory phenotype (SASP). Senescent hepatic stellate cells play a role as cancer-associated fibroblasts (CAFs), showing SASP phenotype.

In addition, lipoteichoic acid (LTA), a gram-positive gut microbial component, enhances SASP, including SASP factor expression as well as COX2-mediated PGE2 production via Toll-like receptor 2 activation (2).

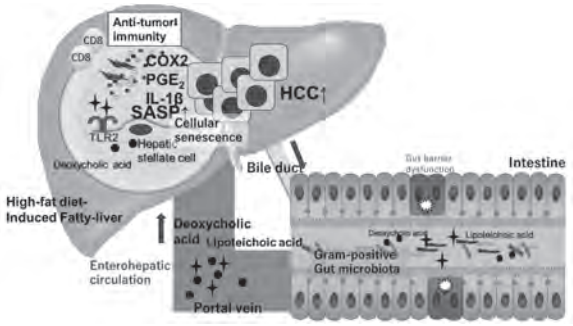

Figure 2 Gut microbiota-derived factors enhance SASP in senescent hepatic stellate cells. Gut microbiota-derived deoxycholic acid provokes cellular senescence of hepatic stellate cells, and lipoteichoic acid (LTA), a bacterial cell wall component, promotes SASP induction, including COX2 expression, a rate-limiting enzyme for PGE2 production, thereby suppressing anti-tumor immunity and promoting tumor progression.

Furthermore, IL-33, which is highly expressed in liver tumor regions, particularly within senescent HSCs (becoming senescent CAFs), plays a crucial role in HCC development in an IL-1β-dependent manner. We found that the SASP factors, including IL-33 and IL-1β, were released via membrane pores formed by the clustering of gasdermin D N-terminal domain, which is facilitated by LTA. The released IL-33 suppresses antitumor immunity by activating ST2-positive Treg cells, thereby contributing to the progression of steatosis-associated HCC (3).

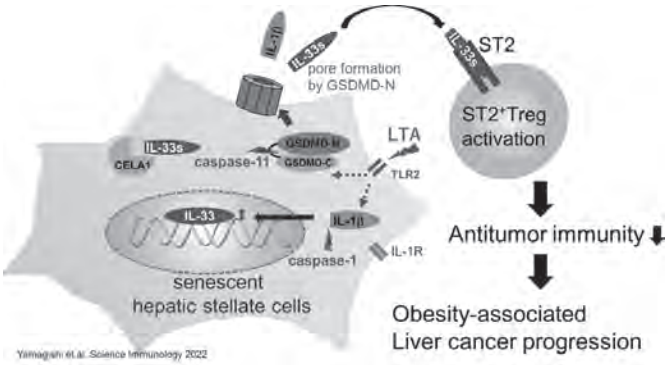

Figure 3 SASP factors are released via the gasdermin D-mediated cell membrane pore. LTA upregulates caspase 11 and triggers the cleavage of gasdermin D to create a pore in the cell membrane. SASP factors such as IL1β and IL-33 are exported via this pore.

We further characterized HSCs as cancer-associated fibroblasts (CAFs) using Flex single-cell transcriptome analysis of human HCC tissues, along with spatial transcriptome analysis showing the localization of each HSC type. We identified a SASP factor from senescent CAFs that correlated with the patients' poor prognosis, highlighting their potential as biomarkers for steatotic HCC progression. I would like to present additional recent findings that further elucidate the immunosuppressive mechanisms driving steatosis-associated HCC.

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METABOLIC DRIVERS OF T CELL DYSFUNCTION IN CANCER

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Immunotherapy has changed the treatment paradigm in cancer, although response rates remain low and relapses are common. It remains difficult to accurately predict patients that will respond to immunotherapy, and patients that fail immunotherapy have few options for continued treatment. However, it is now clear that patients need functional T cells within the tumor bed capable of rejuvenation in order to respond well to immunotherapy.

Unfortunately, the tumor microenvironment (TME) remains a major barrier to immunotherapy response, as it concentrates a number of immunosuppressive mechanisms at the same site. The TME recruits suppressive populations like regulatory T cells, creates physical barriers to infiltration, and produces a metabolically harsh environment through altered vasculature and heightened cellular metabolism. As T cells are chronically activated in this stressful condition, they differentiate down an alternative pathway termed T cell exhaustion. Our lab has been exploring the metabolic contributions to T cell exhaustion, and have revealed that hypoxic stress[1], mitochondrial dysfunction[2], lactate exposure[3], and other nutrient stresses[4] can accelerate, stabilize, and enforce the dysfunctional phenotype of exhausted T cells.

In this talk, I will discuss our newest findings describing how metabolic flux in T cell exhaustion is altered. As T cells become chronically activated, their mitochondrial proteome becomes dramatically altered, shifting from a carbon processing state to a carbon exporting state. A key protein is the mitochondrial citrate carrier (encoded by *Slc25a1*), which transports TCA cycle citrate back to the cytosol. This mitochondrial rewiring induces lipid accumulation, hyperacetylation of the proteome, and altered differentiation to exhaustion. Deletion or inhibition of the citrate carrier results in superior T cell immunity through a

number of complementary mechanisms. Our data provide insight in how to prevent or reprogram exhausted T cells to function in the tumor microenvironment, providing hope for patients with cancer who have failed immunotherapy.

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GUT DYSBIOSIS CAUSE RESISTANCE TO CANCER IMMUNOTHERAPY: A ROLE FOR BILE ACIDS AND SUBCLINICAL CHOLESTASIS

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Immune checkpoint blockade (ICB) efficacy relies on gut microbiota composition. Indeed, over the last decade, we and others unveiled that the composition of the gut microbiota largely governs the anticancer immune response in the context of anticancer chemotherapies and immunotherapies [1-9]. One of the most striking discovery from this work originated from the description of the immunosuppressive role of antibiotics against the immunostimulatory effects mediated by immune checkpoint inhibitors in oncology. Antibiotics (large spectrum, beta- lactams, fluoroquinolones, macrolides, tetracyclines but not vancomycin) administered 1 to 2 months prior to immune checkpoint blockade in advanced cancer patients (stage III, stage IV) have detrimental effects on progression free survival and overall survival across various malignancies (lung, kidney, bladder carcinoma and melanoma) in prospective and retrospective studies (meta-analyses on 42, 000 patients in >100 studies ([4, 10, 11]. Antibiotics are not the only comedications associated with gut dysbiosis since proton pump inhibitors or benzodiazepines also disturb the taxonomic composition of the stools and reduce the efficacy of ICB [12]. Moreover, carcinogenesis by itself is sensed by the ileum [7], triggering a transient mucosal atrophy, characterized by crypt apoptosis, and ectopic proliferation of the enteroendocrine cells expressing tyrosine hydroxylase, culminating in a protracted gut dysbiosis associated with cancer outgrowth. This beta-adrenergic receptor-dependent stress ileopathy [7] is not cancer-specific but observed in many chronic inflammatory disorders[13, 14]. Since then, the team computed 800 metagenomes from cancer patients and defined two species interacting groups of bacteria, SIG1 and SIG2, that anticorrelated with each other and with the prognosis of ICB

-treated patients [15]. We can then calculate the SIG2/SIG1 score for each individual and predict long term response to ICB using shot gun metagenomics or PCR [15].

Dysbiosis, characterised by *Enterocloster clostridioformis* (EC) overrepresentation, notably after antibiotics (ABX) cessation induces a downregulation of the protein MAdCAM-1 expressed on the ileum. MAdCAM-1 binds to the integrin $\alpha 4\beta 7$ expressed on lymphocytes and allows their homing to the gut. The downregulation of MAdCAM-1 induces an exodus of Treg from the gut toward the tumor and leads to exhaustion of tumor infiltrating lymphocytes and resistance to αPD-1 in mice [16]. Soluble MAdCAM-1 can be monitored by ELISA in the serum of patients and is predicting overall survival in several independent cohorts of cancer patients. In addition, sMAdCAM-1 is a proxy of gut dysbiosis, patients with low sMAdCAM-1 harboring oral taxa, and a relative over dominance of the genus Enterocloster [16]. Aside from altering the gut taxonomic composition and the systemic immune tonus, antibiotics affect the holometabolism. We found a dysregulation of plasmatic bile acids (BA) in dysbiotic mice. However, the precise BA landscape associated with intestinal dysbiosis and cancer is unclear as well as its immunological significance. In vivo, gut dysbiosis was induced by ABX or EC treatment. We evaluated ICB response after BA gavages in 2 tumor model (MCA205 and RET). BA were administered 4 times every 2 days starting at day 2 whereas ICB were injected 4 times every 3 days starting at day 5 post -tumor injection. The regulation of MAdCAM-1 expression was assessed by RT-qPCR. BA, such as (glyco)deoxycholic acid ((G)DCA) were tested. We performed metabolomics (MB) analysis in hosts administered with antibiotics or *Enterocloster clostridioformis* (de la Varende AL et al. submitted). In patients, we collected samples from cohorts to detect BA by MB. We also used de-identified clinical data collected from the trials, focusing on gamma glutamyl transferase (γGT). These data were used to explore associations between γGT levels and clinical outcome(de la Varende AL et al. submitted). In mice, ABX and or EC-induced loss of (G)DCA led to resistance to αPD-1. However, restoration of physiological plasma concentrations of these BA after oral feeding could control tumor growth during ICB despite ABX uptake. The mechanism underlying this effect is a recirculation of GDCA to tumor beds that increase polyamines and prevents effector memory T cell exhaustion. GDCA upregulates MAdCAM-1 expression in the ileum thereby blunting the EC-mediated exodus of Tr17 from the gut to the tdLN. In humans, we observed the same BA perturbations post ABX with a loss in secondary and gain in tauro-conjugated BA. Cholestasis is defined by high γGT, (2 upper limit of normal, ULN:M≥45U/L, F≥35U/L), and an increase of BA in the plasma. In 6 cohorts of patients, elevated tauroconjugated BA or high γGT were associated with worse progression free survival (PFS) and overall (OS) (de la Varende AL et al. submitted). Subclinical cholestasis was correlated with prior ABX consumption and gut

dysbiosis monitored by the toposcore. Altogether, these findings unveil for the first time the clinical relevance of a gut-liver axis in cancer distal for the liver and that subclinical cholestasis is a hallmark of gut dysbiosis causing resistance to ICB.

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CONCLUDING REMARKS

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As the chairperson of the organizing committee for the 53rd International Symposium of the Princess Takamatsu Cancer Research Fund, I would like to express my sincere appreciation to all the speakers and discussants—especially those who traveled from overseas—for sharing their cutting-edge research and insights. Your expertise and dedication were instrumental in making this symposium a truly enriching experience. Notably, despite the logistical challenges caused by the U.S. government shutdown, we were able to successfully complete the entire program thanks to the resilience and cooperation of our participants.

Throughout the symposium, we engaged in fruitful and exciting discussions spanning a wide range of critical topics, including: immunosuppressive cells such as regulatory T (Treg) cells; cell metabolism, trafficking, and tumor antigens; vaccines; antigen presentation and co-stimulation; environmental factors such as microbiota and aging; and advanced cell therapies, including tumor-infiltrating lymphocytes (TILs) and chimeric antigen receptor (CAR)-T cell therapies. Additionally, we explored the transformative role of AI in patient stratification and its evolving power as a research tool.

We were honored to have Dr. Jedd D. Wolchok, a pioneer in cancer immunotherapy, deliver the Nakahara Memorial Lecture. His presentation on the clinical development of immune checkpoint inhibitors and combination therapies, as well as his insights into the potential of novel CAR-T therapies, offered valuable perspectives on next-generation treatments.

The keynote lecture further sparked profound discussions on the immune system's role across all stages of cancer—from initiation to metastasis. The concept that genomic abnormalities in cancer cells drive immunosuppression in the tumor microenvironment offers a compelling vision for integrating oncology and immunology into genomic precision medicine.

I would like to extend my deepest gratitude to the organizing committee members and the supporting staff. Their dedication and meticulous efforts were invaluable in fostering productive discussions and innovations.

We are also profoundly grateful to the Princess Takamatsu Cancer Research Fund for its continuous support in advancing cancer research through this symposium. The Fund's commitment to promoting scientific progress and international collaboration is central to our efforts. I am confident that the interactions fostered here will catalyze new research opportunities and contribute significantly to the development of novel cancer immunotherapies.

Thank you once again to everyone who contributed to the success of this symposium. We anticipate the opportunity to continue our collaborative efforts and express our eagerness to welcome you at future meetings.

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