Journal for ImmunoTherapy of Cancer

Volume 14 Supplement 1

ITOC12 - 12TH IMMUNOTHERAPY OF CANCER CONFERENCE
MARCH 12 – 14, 2026 – MUNICH, GERMANY

ABSTRACTS

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March 12 – 14, 2026 – Munich, Germany

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Contributions of up to EUR 10 000.- (Ten thousand Euros, or equivalent value in kind) per year per company are considered "Modest". Contributions above EUR 10 000.- per year are considered "Significant".
If disclosures are missing, there is nothing to declare.

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ABSTRACTS

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Oral Presentations

Plenary Session 3: New Targets and New Leads

O03.04 ANTI-KIR2DL4 STIMULATION ENHANCES THE CYTOTOXIC ACTIVITY OF NK-92 CELLS AGAINST PROSTATE CANCER

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10.1136/JITC-2026-ITOC.1

Background Immunotherapy has become a promising approach in cancer treatment by harnessing the body's immune system to selectively eliminate malignant cells while sparing normal tissues. Natural killer (NK) cells, due to their innate cytotoxicity, play a crucial role in tumor immunosurveillance. Their cytolytic activity depends on the balance between activating and inhibitory receptors. Modulating this balance toward activation enhances anti-tumor potential. Recent studies indicate that anti-KIR2DL4 antibodies function as agonistic agents promoting NK cell activation. However, the effects of anti-KIR2DL4 stimulation on NK-92 cells against DU145 prostate cancer cells remain unexplored.

Methods This study examined the anti-cancer effects of anti-KIR2DL4–stimulated NK-92 cells on DU145 prostate cancer and PNT1A normal prostate epithelial cells. NK-92 cells were pretreated with anti-KIR2DL4 antibodies and co-cultured with target cells at effector-to-target (E:T) ratios of 1:5 and 1:10. Cytotoxicity was measured using the WST-1 assay to determine the optimal E:T ratio. Apoptosis-related proteins including Caspase-3, Caspase-8, Caspase-9, and BAX were analyzed by immunostaining to identify apoptotic pathways activated by anti-KIR2DL4 stimulation.

Results Anti-KIR2DL4–stimulated NK-92 cells demonstrated markedly higher cytotoxicity toward DU145 cancer cells compared with unstimulated controls and normal PNT1A cells. The most potent cytotoxic effect occurred at an E:T ratio of 1:10. Immunostaining revealed significant upregulation of Caspase-3, Caspase-8, Caspase-9, and BAX expression, indicating activation of both intrinsic and extrinsic apoptotic pathways. Importantly, normal prostate epithelial cells exhibited minimal damage, suggesting that this approach selectively targets malignant cells while preserving normal tissue integrity. These results confirm that agonistic stimulation of KIR2DL4 enhances NK-92 cell cytotoxicity and apoptosis induction specifically against prostate cancer cells.

Conclusion Agonistic activation of KIR2DL4 significantly augments NK-92–mediated cytotoxic and pro-apoptotic effects on prostate cancer cells with minimal toxicity to normal prostate cells. This strategy provides a promising and selective immunotherapeutic avenue for prostate cancer treatment. The data offer essential preclinical insights for the development of future translational and clinical applications utilizing KIR2DL4-targeted NK cell therapy. Further in vivo and phase-based investigations are warranted to validate these findings and optimize their therapeutic potential.

O03.05 AGING DRIVES IMMUNOSUPPRESSIVE NICHES IN GALLBLADDER CANCER: SINGLE-CELL AND SPATIAL TRANSCRIPTOMIC INSIGHTS FOR IMMUNOTHERAPY

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10.1136/JITC-2026-ITOC.2

Background Aging profoundly reshapes the tumor microenvironment (TME) and influences the efficacy of cancer immunotherapy. In gallbladder cancer (GBC), age-related alterations in the TME may modulate tumor initiation, immune evasion, and metastatic progression. Although aging has been shown to promote immunosuppressive microenvironments in several malignancies, its impact in GBC remains poorly defined. This study aims to characterize aging-dependent remodeling of the GBC TME by integrating single-cell and spatial transcriptomics.

Methods Tumor specimens from two GBC cohorts— younger patients (<60 years, n = 5) and older patients (>70 years, n = 5)—underwent single-cell RNA sequencing and spatial transcriptomic profiling. We evaluated age-associated differences in cellular composition, transcription factor activity, cell–cell communication, and lineage trajectories. Spatial transcriptomics was further employed to identify aging-specific immunosuppressive ecological niches within the TME and to validate key immunoregulatory pathways.

Results Aging markedly remodels both immune and stromal compartments of the GBC TME. The older cohort showed a substantial increase in immunosuppressive cell populations, including regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). Tregs exhibited enhanced suppressive signatures, while TAMs shifted toward a pro-tumorigenic M2 phenotype. Moreover, aging expanded exhausted $CD8^+$ T cells, collectively attenuating antitumor immunity. Stromal fibroblasts from older patients upregulated extracellular matrix (ECM)–remodeling genes, contributing to a more fibrotic and tumor-promoting environment. Endothelial cells displayed transcriptional alterations indicative of vascular dysfunction. Transcription factor analysis revealed increased NF-κB and STAT3 activation across multiple cell types, whereas pseudotime trajectories suggested aging-driven differentiation toward tumor-supportive states. Importantly, spatial transcriptomics identified a distinct and highly immunosuppressive niche enriched in older patients. This niche was characterized by pronounced NF-κB and STAT3 signaling activity and contrasted sharply with immune-active regions. These findings demonstrate that aging not only alters cellular proportions but also reorganizes the spatial architecture of immunosuppression in GBC.

Conclusion Aging induces profound immunosuppressive remodeling in the GBC TME, weakening antitumor immunity and potentially reducing responsiveness to immunotherapy. The identification of aging-specific immunosuppressive niches highlights new opportunities for therapeutic intervention. Our findings underscore the need for age-tailored immunotherapeutic strategies and provide mechanistic insights into optimizing treatment for elderly GBC patients.

Plenary Session 11: Advances in Clinical Immunotherapy of Multiple Myeloma – Part II

O11.02 β-ADRENERGIC BLOCKADE UNLEASHES CYTOTOXIC CD4 T CELL IMMUNITY IN CANCER

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10.1136/JITC-2026-ITOC.3

Background β-adrenergic signaling has been implicated in tumor progression and immune suppression, and epidemiological and clinical observations suggest potential anti-cancer benefits of β-blockers. Propranolol, a widely prescribed nonselective β-blocker, has recently demonstrated clinical efficacy in vascular sarcomas; however, the anti-tumorigenic mechanisms underlying its anti-tumor and anti-metastatic effects remain poorly defined.

Materials and Methods We investigated the impact of propranolol in multiple syngeneic murine cancer models, including multiple subcutaneous tumor models as well as experimental and spontaneous metastasis models. Tumor growth, metastatic burden, survival, and therapeutic interactions with immune checkpoint inhibitors were assessed. Immune cell dependencies were determined using antibody-mediated cell depletion and transgenic mice enabling specific cell type depletion. Tumor and systemic immune landscapes were characterized by flow cytometry and RNA sequencing of FACS-sorted immune populations.

Results Propranolol treatment significantly delayed primary tumor growth, reduced metastatic dissemination, and improved survival across models. Through depletion of specific immune cell populations, we uncovered a striking and unexpected dependency on CD4 T cells. Propranolol-mediated control of metastasis was entirely independent of CD8 T cells and NK cells but required CD4 T cells, revealing a previously underappreciated mechanism of β-adrenergic regulation of anti-tumor immunity. Mechanistically, propranolol induced a Th1-polarized, cytotoxic CD4 T cell program characterized by enhanced effector function and tumor control, which required MHC class II expression by cancer cells for full activity. The induction of a cytotoxic CD4 T cell phenotype was confirmed in ex vivo cytotoxicity assays. In parallel, propranolol reshaped the myeloid compartment by reducing intratumoral monocytic myeloid-derived suppressor cells (mMDSCs). Strikingly, depletion of monocytes also abrogated the therapeutic efficacy of propranolol, highlighting critical myeloid-CD4 T cell crosstalk. Importantly, propranolol synergized strongly with anti-CTLA-4 therapy, leading to enhanced $CD4^+$ T cell infiltration and superior control of both primary tumors and metastases.

Conclusion Our findings identify β-adrenergic signaling as a key suppressor of cytotoxic CD4 T cell-mediated anti-tumor immunity and metastasis control. Given its favorable safety profile and clinical availability, propranolol represents a highly translatable immunomodulatory agent that may enhance ICI efficacy.

Plenary Session 12: Young Researcher Session

O12.01 INCREASED TECLISTAMAB DOSING INTERVAL IMPROVES CLONOTYPIC DIVERSITY AND REDUCES INFECTION RISK IN MULTIPLE MYELOMA

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10.1136/JITC-2026-ITOC.4

Background Bispecific antibodies are among the most effective treatments for relapsed/refractory (RRMM) multiple myeloma. However, most approved agents, including teclistamab, were developed with short dosing intervals and indefinite treatment duration. In view of the rapid and deep responses in most patients, the rationale for continuous dose-dense therapy is limited. Concurrently, infectious complications remain a major challenge, requiring treatment breaks and leading to fatal outcomes. In clinical practice, dose interval extension, from weekly to biweekly or monthly, has become increasingly common.

Still, the immunological impact of extended interval dosing remains poorly understood. To address this, we conducted a multiomics study comparing immune effects of weekly (q1w), biweekly (q2w), and monthly (q4w) teclistamab dosing.

Material and Methods We biobanked peripheral blood mononuclear cells (PBMCs, n=87) from 30 RRMM patients who switched from q1w (n=34) to q2w (n=12) and/or q4w (n=33) teclistamab dosing. As a control, 8 baseline samples were also collected prior to treatment initiation.

To characterize immune changes across dosing intervals, we performed single-cell multimodal sequencing (scRNA/CITE/TCR; n=28 (16 patients)) and 14-color flow cytometry (n=68). Additionally, we conducted in vitro cytotoxicity assays using MM.1S target cells and patient-derived T cells (n=20) across varying effector-to-target ratios.

Results We first evaluated infection rates across treatment schedules dosing after 12 months and observed a significantly lower incidence of infections with better progression-free survival in patients receiving q4w compared to q1w.

Next, from flow cytometry analysis, we observed elevated leukocytes count along with a higher number of T cells in the q4w samples compared to the q1w samples. ScRNA/CITE-seq analysis of T cells showed no significant differences in exhaustion signature across dosing schedules, a finding that was confirmed by flow cytometry. Furthermore, we detected increased frequencies of naïve T cells and central memory T cells, consistent with partial T-cell recovery during longer treatment-free intervals. From in vitro cytotoxicity assays, no significant differences in killing capacity were observed between T cells from patients on q1w, q2w or q4w dosing schedules.

scTCR sequencing revealed pronounced T-cell receptor clonotypic diversity in q4w compared to q1w samples. Mapping

TCR sequences to known viral epitopes exhibited a restricted antiviral repertoire in q1w samples. In contrast, q4w samples showed significantly broader viral TCR diversity, potentially contributing to the lower infection rate observed in this group. Lastly, we applied a tumor reactivity score (Kehl et al., Blood, 2025). From which, MM-reactive T cells markedly appeared to be increased from baseline to q1w dosing as expected, followed by a modest, non-significant decline with q2w and q4w intervals.

Conclusion Our data support an initial dose-dense treatment phase to expand tumor-reactive T cells, followed by extended dosing intervals in responders to promote immune recovery, restore T-cell diversity, and reduce infections, while maintaining anti-myeloma activity.

Nazia Afrin: None; Florian Eisele: Sobi, AbbVie; Emilia Stanojkovska: None; Mara John: None; Hanna Fischer: None; Moutaz Helal: None; Carolin Köchel: None; Niklas Kehl: None; Christine Riedhammer: Takeda; Sofie-Katrin Kadel: Jannsen; Julia Mersi: None; Torsten Steinbrunn: None; Johannes M. Waldschmidt: None; Sabrina Kraus: None; Johannes Duell: Novartis, Gilead/Kite, BMS, Janssen, Abbvie, Beigene; K. Martin Kortüm: BMS, Menarini, Novartis, Pfizer, GSK, J&J, AbbVie; Mirco Friedrich: None; Hermann Einsele: Janssen, Bristol-Myers Squibb; Angela Riedel: None; Leo Rasche: Janssen, Bristol-Myers Squibb

O12.02 ERBB2 SIGNALING DRIVES IMMUNE CELL EVASION AND RESISTANCE AGAINST IMMUNOTHERAPY IN SMALL CELL LUNG CANCER

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10.1136/JITC-2026-ITOC.5

Background Small cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers and is characterized by its highly aggressive phenotype and its dismal outcome. Although the addition of immune checkpoint blockade to carboplatin and etoposide treatment has improved outcome in SCLC patients, SCLC cells finally acquire the ability to evade immunosurveillance and develop resistance against immune checkpoint blockade.

Methods To investigate molecular mechanisms underlying immune evasion and metastasis in SCLC, we performed an integrated analysis of human and murine SCLC specimens and combined high-dimensional profiling with functional validation. Initial analysis of single-cell RNA sequencing (scRNA-seq) data revealed differentially expressed genes between primary and metastatic lesions. Loss of MHC-I in SCLC metastasis was validated in matched human and murine samples of primary and metastatic SCLC with flow cytometry and immunohistochemistry. We utilized mass spectrometry and phospho-tyrosine kinase arrays to identify signaling pathways involved in immune regulation. Functional validation of ERBB2 as relevant regulator was performed using genetic and pharmacological approaches in vitro and in autochthonous murine SCLC models. The therapeutic efficacy of combined ERBB2 inhibition and immunotherapy was assessed in autochthonous SCLC mouse models through survival analysis, single-cell and TCR sequencing and flow cytometry.

Results Analysis of scRNA-seq and matched human samples revealed that metastatic SCLC exhibits a loss of MHC-I expression, suggesting a role in metastasis formation and immune evasion. Genetic knockout of MHC-I in SCLC cells markedly reduced immune cell infiltration and promoted metastasis formation in immunocompetent mouse models. We identified ERBB2 as a negative regulator of MHC-I expression and a promoter of immune-modulatory transcripts. Genetic or pharmacologic inhibition of ERBB2 restored MHC-I expression and immunogenic signaling pathways. In autochthonous SCLC mouse model ERBB2 inhibition could significantly reduce metastasis formation. Notably, the combination of ERBB2 blockade with PD-1 inhibition produced synergistic anti-tumor responses, overcoming MHC-I loss and enhancing immune infiltration resulting in deep remissions and substantial extension of survival.

Conclusion Our study identifies ERBB2 signaling as a critical driver of immune evasion and metastasis in SCLC through downregulation of MHC-I. Targeting ERBB2 restores MHC-I expression, enhances immune infiltration, and synergizes with PD-1 blockade to elicit robust anti-tumor responses. These findings reveal a therapeutically actionable pathway for overcoming immunotherapy resistance in SCLC and provide a strong rationale for combining ERBB2 inhibition with immune checkpoint therapy in this aggressive malignancy.

O12.03 MENIN INHIBITION FOLLOWING ALLO-HCT ENHANCES GRAFT-VERSUS-LEUKEMIA EFFECTS THROUGH ENDOGENOUS RETROVIRUS INDUCTION IN AML AND T-CELL ACTIVATION

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10.1136/JITC-2026-ITOC.6

Background Acute myeloid leukemia (AML) with chromosomal rearrangements involving the lysine methyltransferase 2A (KMT2A) gene and mutations in the nucleophosmin (NPM1) gene often relapse following allogeneic hematopoietic cell transplantation (allo-HCT). Targeting the interaction between menin and KMT2A with pharmacological menin-inhibitors disrupts oncogenic KMT2A chromatin complexes, impairing abnormal self-renewal and promoting myeloid differentiation. However, its impact on post-transplant immune surveillance and graft-versus-leukemia (GVL) activity has not been defined. We investigated whether menin inhibition modulates leukemia immunogenicity and donor T-cell function to enhance GVL effects.

Methods Human AML cell lines and primary samples were treated with menin inhibitors. MHC expression, cytokines, and cytolytic markers were analyzed by flow cytometry. Human endogenous retrovirus (HERV) and interferon-stimulated gene (ISG) expression were assessed by qRT-PCR and RNA-seq, while chromatin accessibility and menin/KMT2A occupancy were examined by ATAC-seq and ChIP-seq. Primary human T-cells were activated in the presence or absence of menin-inhibitors, and activation, exhaustion, cytokine secretion, and cytotoxicity were evaluated by (high-dimensional) flow cytometry, ELISA, and killing assays. GVL activity was assessed in allogeneic and xenogeneic AML transplantation models, and GVHD was evaluated by histopathology and inflammatory marker analysis.

Results Menin inhibition consistently induced CIITA and MHC class II expression in KMT2A-rearranged and NPM1-mutated AML cell lines and primary patient samples, both in vitro and in vivo. Increased MHC-II expression significantly enhanced susceptibility of AML cells to allogeneic T-cell–mediated cytotoxicity. This effect was preserved in xenograft models using primary human AML, resulting in improved leukemia control and prolonged survival after T-cell transfer.

Mechanistically, menin inhibition derepressed multiple families of human endogenous retroviruses, leading to accumulation of double-stranded RNA and activation of a type I interferon response. This was accompanied by robust upregulation of interferon-stimulated genes and increased chromatin accessibility at immune-related loci. Pharmacologic inhibition of the cGAS-STING pathway reversed ISG induction and MHC-II upregulation, demonstrating that this signaling pathway is required for menin inhibitor–mediated immunogenic remodeling.

In parallel, menin inhibition directly enhanced donor T-cell effector function. Treated T-cells showed increased production of TNF-α, IFN-γ, perforin, and granzyme A/B, alongside reduced expression of inhibitory receptors and exhaustion-associated markers. Chromatin immunoprecipitation and transcriptional profiling revealed reduced menin-KMT2A occupancy at genes encoding negative regulators of T-cell activation. Functionally, menin-treated T-cells exhibited increased cytolytic activity against AML targets in vitro and in vivo.

Importantly, enhanced GVL activity did not translate into increased GVHD. Histopathological analysis, inflammatory cytokine expression in target organs, and body weight monitoring showed no exacerbation of acute GVHD in menin inhibitor–treated mice.

Conclusion Menin inhibition amplifies GVL through two converging mechanisms: induction of leukemia immunogenicity via a HERV–interferon–MHC-II axis and direct enhancement of donor T-cell cytotoxicity. These findings support menin inhibition as a rational post-transplant maintenance strategy to prevent AML relapse without increasing GVHD risk.

V.Fetsch: none, L.Schwöbel: none, E.Ozyerli-Goknar: none, A.Stell: none, M.Punta: none, T.Plenge: none, T.Klaus: none, M.K.Gupta: none, G.Andrieux: none, K.Shoumariyeh: none, S.Pfeiffer: none, E.Corrales: none, L.Schlenke: none, H.Baniadam: none, S.M.Brandl: none, M.Andreis: none, M.Remen: none, A.Hartmann: none, K.Grueter: none, M.Zwick: none, N.Köhler: none, M.Kuban: none, E.Metzger: none, C.Rummelt: none, J.Duyster: none, M.Boerries: none, M.Hofmann: none, J.Färber: none, L.Braun: none, A.J.Zähringer: none, M.Lübbert: none, C.Toffalori: none, L.Vago: none, F.H.Heidel: Merck, Janssen, Kartos, GSK, Abbvie, Silence Therapeutics, Prelude Therapeutics, AOP Health, BMS/Celgene, Novartis: modest, S.Minguet: none, P.Apostolova: none, T.Feuchtinger: none, K.Maas-Bauer: none, F.Blaeschke: none, M.W.M.Kühn: AbbVie, Bristol Myers Squibb/Celgene, Gilead, Johnson & Johnson, Jazz, Pfizer, Servier, Kura Oncology, Syndax Pharmaceuticals Inc: modest, H.Th.M.Timmers: none, T.Wertheimer: none, F.Perner: Syndax Pharmaceuticals, CHARM Therapeutics: modest, R.Zeiser: Novartis, Incyte, Sanofi, Medac, Neovii, Mallinckrodt: modest.

O12.04 A MODULAR CAR T CELL PLATFORM FOR TARGETED IMMUNOTHERAPY OF ACUTE MYELOID LEUKEMIA

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10.1136/JITC-2026-ITOC.7

Background CAR T cell therapy has shown limited efficacy in acute myeloid leukemia (AML), largely due to genetic and molecular heterogeneity and the emergence of antigen-negative relapse. Adapter CAR T cells can overcome key limitations in AML. Anti-P329G adapter CAR T cells recognize the P329G mutation within the Fc region of Fc-silenced human IgG1 antibodies carrying L234A/L235A mutations and thereby represent a disease agnostic and modular cell therapy platform. The advantage of such P329G-containing antibodies lays in their clinical validation against multiple tumor antigens. These antibodies as adaptor modules are therefore readily available for combination with universal anti-P329G adapter CAR T cells.
Methods ScFv-based 2nd generation anti-P329G CAR T cells were combined with P329G-Fc-mutated antibodies targeting the established AML-related target antigens CD33 and CD123 as well as the newly identified target CSF1R. Conventional, scFv-matched CAR T cells targeting the same antigens were used as positive controls. Untransduced or anti-CD19 CAR T cells served as negative control. Target antigen expression was analyzed on cell lines and primary patient samples (peripheral blood, bone marrow). Immunophenotyping and proliferation measurement of anti-P329G CAR T cells was performed using flow cytometry. Effector functions were evaluated against different wildtype and antigen knockout (KO) AML cell lines and primary AML blasts by ELISA-, flow cytometry- and luciferase-based analysis. Effector function in an antigen-escape setting was evaluated in vitro using IncuCyte live cell imaging. Confocal imaging of anti-P329G CAR T cell-tumor cell interaction was performed. In vivo functionality was confirmed in immunodeficient mice after injection of THP-1 and MV4-11 cells.
Results AML cell lines and primary AML blasts could be successfully targeted by anti-P329G CAR T cells combined with P329G-Fc-mutated CAR-adapter molecules targeting CD33, CD123 and CSF1R. Effector functions of anti-P329G CAR T cells were equivalent to conventional CAR T cells. Differences in efficacy were depending on the expression profile of target antigens. The platform demonstrated efficient in vitro activation, proliferation and cytotoxicity. Co-culture assays using antigen KO cells and protein stimulation assays confirmed that anti-P329G CAR T cells operated in a modular, antigen-specific and reversible manner and enabled both combinatorial and sequential targeting of AML. Live-cell imaging based serial cytotoxicity assays employing re-challenge with antigen knockout cell lines showed that targeting antigen escape by switching of the binder antibody is feasible. In immunodeficient mice, growth of THP-1 or MV4-11 tumor cells could be successfully controlled and eradicated by anti-P329G CAR T cells.
Conclusion Combination of anti-P329G CAR T cells with P329G-Fc-mutated CAR-adapter molecules mediated robust effector functions across multiple AML models when directed against CD33, CD123 and CSF1R. The platform enables modular, combinatorial and sequential targeting of distinct antigens, providing a flexible platform with the potential to overcome key limitations of CAR T cell therapy in AML.

K. Gabriel: none S. Stock: none A. Hauptstein: none T. Strzalkowski: none L. Rohrbacher: none A. Gottschlich: Tabby Therapeutics, immuno-oncology patents (all significant) J. Dörr: none V. D. Menkhoff: none S. Nandi: none G. Hänel: none C. Carcopino: none D. Simnica: none M. Surowka: Roche previous employment and stock ownership (significant) P. Bruenker: Roche previous employment and stock and patent ownership (significant) D. Darowski: Roche employment and stock and patent ownership (significant) S. Endres: none M. von Bergwelt-Baildon: M. Subklewe: AbbVie, Amgen, Molecular Partners, Pierre Fabre, Roche (all significant) C. Klein: Roche previous employment and stock and patent ownership (significant) S. Kobold: Miltenyi, Galapagos, Cymab, Novartis, Plectonic, TCR2 Inc., BMS, GSK, Carina Biotech, Tabby Therapeutics, Catalym GmbH, Plectonic GmbH, and Arcus Bioscience, several immuno-oncology patents (all significant)

Plenary Session 13: Immunooncology in Gynecologic Oncology

O13.03 CANCER CELL–INTRINSIC REWIRING OF THE SECRETOME IN IMMUNE-EXCLUDED COLORECTAL CANCER

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10.1136/JITC-2026-ITOC.8

Background Despite the key role of secreted factors in coordinating anti-tumor immunity, our understanding of how cancer cells rewire their secretome to avoid immune destruction is still very poor. This is largely due to technological shortcomings that limit our ability to reliably and comprehensively profile the cancer cell-intrinsic secretome of human tumors.
Methods Here, we develop a click chemistry-based approach to selectively profile the secretome of patient-derived colorectal cancer (CRC) and healthy colon (HC) organoids with high resolution. We use THRONCAT[1] to label the nascent proteome through incorporation of the threonine analog β-ethynylserine. This allows selectively isolating newly synthesized secreted proteins followed by their characterization using mass spectrometry. Focusing on immunologically unresponsive microsatellite stable (MSS) cancers, we compare the secretome of CRC

and HC organoids to determine how cancer cells rewire their secretome, in samples directly derived from patient tissues. We integrate our secretomics datasets with clinical bulk and single cell RNA sequencing data to identify CRC-specific secreted proteins that mark immune excluded tumors.

Results We observe a major rewiring of the secretome in the transition to malignancy, with 12-27% of all secreted proteins significantly altered between HC and CRC organoids. Notably, the secretome is markedly distinct between the immunologically divergent microsatellite stable (MSS) and instable (MSI) CRC subtypes, with NOTUM emerging as the top differentiating secreted protein. Cross-referencing our secretomics data with clinical datasets reveals that NOTUM expression is associated with decreased T cell infiltration across four independent datasets. In addition, we identify AZGP1 as a CRC-specific secreted factor associated with decreased infiltration of gamma delta T cells.

Conclusion Overall, our study provides an in-depth characterization of the cancer cell-intrinsic secretome of MSS-CRC, and nominates NOTUM and AZGP1 as secreted factors that mark immune excluded tumors. This provides a framework to investigate the cell-intrinsic and -extrinsic regulators of secreted factors of interest to reveal novel immunotherapeutic targets.

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O13.04 THE COMBINATION OF INNATE IMMUNITY, ACTIVATING TUMOR ASSOCIATED MACROPHAGES AND ADAPTIVE IMMUNITY, A PROMISING THERAPEUTIC APPROACH IN HEAVILY TREATED PLATINUM RESISTANT OVARIAN CANCER PATIENTS

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10.1136/JITC-2026-ITOC.9

Background High grade serous or endometrioid platinum-resistant ovarian cancer patients (PROC) who received ≥4 prior chemotherapy lines represent a high unmet medical need, with a median overall survival (OS) of 8–12 months.[1] These patients are resistant to prior chemotherapies and have accumulated treatment-related toxicities. Recently developed ADCs have not demonstrated benefit in PROC patients beyond three prior lines, underscoring the need for new safe and effective therapeutic options.

NI-1801 is a human mesothelin (MSLN) x CD47 bispecific antibody developed to selectively engage CD47 on MSLN+ tumor cells. CD47 blockade enhances innate-cell driven tumor cell phagocytosis through blockade of CD47-SIRP-α interactions and adaptive immune responses through antigen presentation. Combination with anti-PD-1 antibodies can enhance both adaptive and innate immune responses through blockade on activated T cells and PD-1 in tumor associated macrophages.[2] Preliminary safety and efficacy data on PROC patients treated with NI-1801 monotherapy or in combination with pembrolizumab was presented at ESMO 2025 (abstract no 1512O).

Methods 21 patients have been treated with NI-1801 at doses of 600mg and 900 mg in combination with 400 mg pembrolizumab every 6 weeks. Data cutoff: 26th November 2025.

Results Twenty-one patients were treated with NI-1801 at doses of 600 mg and 900 mg in combination with pembrolizumab. At the data cutoff, the median survival follow-up was 16.1 months, with 3 patients ongoing and 12 alive. This regimen has been safe and well tolerated by patients. The overall response rate (ORR) was 28.6% and the clinical benefit rate (ORR+SD) was 47.6%. The PFS rate at 12 months was 22.2% and the OS rate at 18 months was 51.8%. In patients that experienced clinical benefit (10 /21), PFS rate at 12 months was 46.7% and at the time of drafting this abstract the median OS was not reached, immature OS rate at 18 months was 67.5 %. Consistent with observations in other solid tumors treated with immunotherapy, ORR and PFS correlated with OS in this cohort.

Conclusion Combination of innate and adaptive immunity, blocking CD47 and PD-1 is a promising and safe therapeutic option for PROC patients that have received more than 3 chemotherapy lines, a fragile population of patients with ovarian cancer where chemotherapies and ADCs have not demonstrated clinical benefit. This study showed that surrogate endpoints for OS like ORR or PFS that were not predictive in PROC patients treated with chemotherapy, are predictive when treating PROC patients with this combination of innate and adaptive immunotherapies.

Clinical trial information NCT05403554

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The author is an employee of Light Chain Bioscience, the sponsor of the study (NCT05403554). No other conflicts of interest are declared.

Plenary Session 15: CAR- and TCR-engineered T cells

O15.03 MYELOID-T CELL THERAPY INTERACTIONS RESHAPE THE TUMOR MICROENVIRONMENT TO SUSTAIN TUMOR CONTROL

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10.1136/JITC-2026-ITOC.10

T cell–based immunotherapies have transformed cancer treatment, yet their efficacy remains constrained by limited T cell infiltration, rapid exhaustion, and high toxicity rates due to excessive endogenous immune activation, all of which involve the tumor microenvironment.

We reasoned that understanding how therapeutic T cells physically engage with and reprogram the TME is essential for improving treatment outcomes. Here, we combined an immunocompetent adoptive T cell therapy model (EG.7-OVA and ex vivo activated antigen specific T cells) with uLIPSTIC, a genetically encoded enzymatic-labeling system, to directly map intercellular contacts between therapeutic T cells and TME immune populations in vivo. In this system, a donor cell expresses SrtA,

which catalyzes the transfer of a biotinylated LPETG substrate onto a G5 tag in an acceptor cell, which can be detected by flow cytometry. We took advantage of this system by using OT-I SrtA+ mice as T cell donors, and Cd40G5/G5 mice as tumor bearing hosts.

At 72 hours post-therapy, flow cytometry revealed profound remodeling of the myeloid compartment, including expansion of inflammatory Ly6Chigh macrophages. Multiplex cytokine profiling of antigen restimulated tumor lysates showed increased production of GM-CSF, CCL3, CCL4, CCL5, and pro-inflammatory mediators linked to myeloid recruitment and activation. uLIPSTIC labeling demonstrated that OT-I cells formed direct physical contacts with multiple macrophage and dendritic cell subsets. Using EL4 tumors lacking OVA, labeling was largely lost, indicating antigen dependence. To test whether cross-presentation drives these interactions, we generated mixed bone marrow chimeras containing Cd40G5/G5 B2m+/+ and Cd40G5/G5 B2m–/– cells; showing stronger labeling of the H2-Kb^{+} myeloid compartment but an incomplete abrogation of labeling in the B2M-/- deficient compartment, suggesting subsequent antigen independent interactions after an initial priming event.

To capture this interplay with deeper resolution, we performed single cell RNA sequencing coupled with CITE-seq on sorted OT-I and myeloid cells. Therapy induced distinct inflammatory macrophage and dendritic cell states marked by strong proinflammatory and interferon signatures. Trajectory analysis suggested an in situ reprogramming of TME infiltrating populations upon therapy. Assignment of the barcoded anti-biotin to specific clusters allowed us to characterize the transcriptional status of myeloid cells who engaged in interactions with therapeutic T cells, including a Nos2+ macrophage subset, mreg-DCs, and ISG DCs. Finally, conditional depletion of myeloid populations with DTR hosts or treatment with myeloid-depleting antibodies impaired tumor clearance, demonstrating their functional support of T cell therapy.

Collectively, this work provides a mechanistic map of antigen-driven T cell–myeloid crosstalk and identifies myeloid remodeling as a critical determinant of adoptive T cell therapy efficacy.

O15.04 METABOLIC REPROGRAMMING ENHANCES STEM-LIKE AND FUNCTIONAL PROPERTIES OF LUNG CANCER-DERIVED TILS

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10.1136/JITC-2026-ITOC.11

Background Lung cancer remains the leading cause of cancer-related death, with outcomes limited by late-stage diagnosis and resistance to current therapies. Adoptive cell therapy using tumour-infiltrating lymphocytes (TILs) offers durable responses in some solid tumours, but efficacy in lung cancer is constrained by T-cell exhaustion and metabolic dysfunction. Enhancing metabolic fitness is therefore critical to improve TIL-based immunotherapy. Nicotinamide riboside (NR), a nicotinamide adenine dinucleotide (NAD^{+}) precursor that supports mitochondrial function, may restore T-cell energy balance and reduce exhaustion. This study aimed to optimise expansion of lung cancer-derived TILs with enhanced metabolic resilience, stem-like properties, and anti-tumour functionality.

Methods Following written informed consent and ethical approval, tumour tissue from lung cancer patients was processed to generate TILs. Tumour fragments (1–2 mm^2) were cultured for 15 days in interleukin-2 (IL-2)-supplemented medium with or without NR to assess effects on TIL expansion and metabolic fitness. Expanded cells were phenotyped by flow cytometry for CD3, CD4, CD8, CD39, CD69, PD-1, LAG-3, and TIM-3. Functional capacity was evaluated by cytokine secretion (IFN-γ, TNF-α, IL-2, granzyme B) following CD3/CD28/CD137 stimulation or autologous tumour co-culture. SIRT1 and C1QBP expression were quantified by quantitative PCR as markers of NR-induced mitochondrial and metabolic enhancement.

Results TILs were successfully isolated and expanded from 53 lung cancer specimens, demonstrating feasibility of a robust lung TIL manufacturing workflow. Cultures with superior proliferation showed higher proportions of $CD4^{+}$ T cells and an enrichment of stem-like $CD39^{-}CD69^{-}CD8^{+}$ subsets, indicating that helper support and a less differentiated $CD8^{+}$ phenotype favour expansion potential. Expanded TILs retained strong tumour reactivity, secreting high levels of IFN-γ after CD3/CD28/CD137 stimulation or autologous tumour co-culture. Elevated LAG-3 expression correlated with reduced proliferation. NR supplementation further improved TIL quality by increasing stem-like $CD8^{+}$ subsets, reducing PD-1 expression, upregulating SIRT1 and C1QBP, and enhancing secretion of TNF-α, IL-2, and IFN-γ, compatible with improved metabolic and effector function.

Conclusion A clinically relevant process for isolating and expanding lung cancer-derived TILs has been established, aligning with current efforts to advance cellular therapies in solid tumours. Metabolic reprogramming with NR promotes a less differentiated, metabolically resilient, and tumour-reactive TIL phenotype, supporting NR-supplemented TIL products as a promising strategy to achieve more durable and effective cell therapy for lung cancer.

O15.05 DEVELOPMENT OF PEPTIDE-CENTRIC CARS TARGETING IGFBPL-1 FOR CANCER IMMUNOTHERAPY IN SMALL-CELL LUNG CANCER

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10.1136/JITC-2026-ITOC.12

Background Targeting intracellular antigens has to date been impractical for most immunotherapies, due in part to limited accessibility and difficulty identifying reactive T cell receptors (TCRs). However, artificial intelligence (AI) derived de novo peptide design has challenged this paradigm, for the first-time allowing rational design of binders specific for peptide-major histocompatibility complexes (pMHC), which can be utilised as antigen-binding moiety in peptide centric CAR T cells.[1]

Material and Methods To exploit these new capabilities, we have identified an HLA-A*02 restricted peptide derived from IGFBPL-1 (Insulin-Like Growth Factor-Binding Protein 1)

frequently expressed in SCLC (small cell lung cancer). Initial pMHC binder candidate designs were generated using RFdiffusion,[2] followed by a custom-made iterative refinement algorithm. To experimentally identify specific binders, we implemented a high-throughput library screening strategy utilising mammalian cell surface expression. Positive cells were sorted via HLA-A*02-IGFBPL-1 tetramer staining, with enriched binders identified via next-generation sequencing. These screening hits were incorporated as binding moieties of peptide-centric CARs for functional validation in primary human T cells.

Results We achieved an initial binder design success rate of <1%, improving to 2.9% post iterative refinement. Top binder candidates were successfully integrated and transduced into primary human T cells. IGFBPL-1 peptide-centric CARs demonstrated significant functional activity upon co-culture with IGFBPL-1 peptide-pulsed HLA class I-matched target cells, assessed through target killing, cytokine release and expansion. We further demonstrated target specific activation of our IGBFPL-1 peptide-centric CARs against endogenous expression of IGFBPL-1 peptide on multiple HLA-A*02 SCLC cell lines.

Ongoing efforts aim to further characterize IGFBPL-1 peptide centric CAR functionality in vitro and in vivo, with optomisation continuing in both the refinement of the IGFBPL-1 binders and CAR receptor construct pairing.

Conclusion This work presents a significant advance in targeting previously inaccessible intracellular oncoproteins with CAR T cells in SCLC. It also exemplifies the comparative speed and cost-effectiveness of the rational design of peptide centric binders, expanding the diversity of targets accessible to researchers.

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Poster Presentations

Poster Session 1. Cancer Vaccines

P01.01 AUTOLOGOUS LIVE CELL VACCINATION PROLONGS SURVIVAL IN IMMUNOTHERAPY-RESISTANT GLIOMA MODELS

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10.1136/JITC-2026-ITOC.13

Background High grade gliomas remain refractory to immunotherapy. Novel approaches are required to generate a durable immune response. We developed a live cell vaccination strategy and used this approach to reverse engineer the critical components of a curative anti-glioma immune response.

Methods Therapeutic strategies were evaluated in the immunotherapy-resistant SB28 and CT2A glioma models. Glioma cells were implanted intracranially. For peripheral live cell vaccination, additional glioma cells were implanted subcutaneously in the flank, and subjects were treated with immune checkpoint blockade (anti-PD1, anti-CTLA4). To determine the necessary properties of the vaccine, modifications were made to the tumor cells prior to flank implantation. To determine the necessary properties of the immune response, transgenic models, antibody-based depletions, and surgical procedures were utilized to disrupt the endogenous immune response. To replicate the clinical standard of care, intracranial tumors were surgically resected on Day 16 post-implantation, processed, and re-implanted as an autologous vaccine.

Results Peripheral live cell vaccination rendered previously resistant intracranial tumors highly sensitive to dual checkpoint therapy, achieving >80% long-term survival with durable protective immunity against rechallenge without further therapy. Only viable tumor cells—not cell lysates, fixed cells, or heat-killed preparations—conferred protection, and live cells remained effective even when vaccination was delayed 6 days post-intracranial implantation. Therapeutic efficacy was associated with increased CD8+ T cells and reduced myeloid populations, such as Arg1+ monocytes and macrophages, in the tumor microenvironment. Depletion of CD8+ T cells, but not CD4+ T cells or NK cells, abrogated protective responses. Deficiency of conventional type 1 dendritic cells in IRF8+32kb−/− mice also disrupted anti-glioma immunity. Cervical lymph nodes have been thought to play a critical role in intracranial anti-tumor responses, but resection of both superficial and deep cervical lymph nodes failed to disrupt anti-glioma immunity in both primary disease and rechallenge. Tumor MHCI expression has also been thought to be critical. CRISPR-based knockout of β2-microglobulin, which is required for MHCI expression, revealed that tumor MHCI expression was dispensable in the peripheral vaccine for generation of immunity but required in the intracranial tumor for response to the vaccine. Finally, standard of care for patients with gliomas includes surgical resection. Translating this to our models, intracranial tumors were resected, processed and re-implanted in the flank. This autologous live cell vaccination significantly prolonged survival.

Conclusion These results demonstrate that durable anti-glioma immunity is achievable with autologous live cell vaccination in translatable surgical resection models and reveal critical mechanistic insights to guide development of novel immunotherapies.

P01.02 IMPROVING ANTITUMOR IMMUNITY THROUGH INNATE IMMUNE PATHWAY MODULATION: MODIFIED 3P-RNA SHAPES CYTOKINE AND CELL DEATH INDUCTION IN HUMAN AND MURINE MODELS

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10.1136/JITC-2026-ITOC.14

Background Current immunomodulatory antitumor strategies largely target the adaptive immune system; however, efficacy is often limited by a strongly immunosuppressive tumor microenvironment. Therefore, we investigated whether activating innate RNA-sensing pathways can improve antitumor responses. 5′-triphosphate double-stranded RNA (3p-RNA) activates key antiviral dsRNA sensors that regulate cytokine production and cell death. These include retinoic acid-inducible gene I (RIG-I), which is essential for type I interferon (IFN-I) and proinflammatory cytokine induction; the 2′-5′-oligoadeny-

late synthase (OAS) family with RNase L; and protein kinase R (PKR), which promote cell death via translation inhibition.

Recently, we demonstrated that a short 20-bp 3p-RNA induces apoptosis in two steps, where RIG-I primes the cell and OAS1 drives cell death. We hypothesized that modifying 3p-RNA characteristics can differentially activate the RIG-I-mediated cytokine axis and the OAS/PKR-mediated cell-death axis, thereby deciphering ligand preferences of these receptor systems.

Methods We generated a panel of in vitro-transcribed 3p-RNAs of increasing lengths designed to preferentially activate (i) RIG-I alone, (ii) RIG-I plus OAS/RNase L, or (iii) PKR. Human and murine tumor cell lines, including CRISPR knockout lines targeting individual axes (RIG-I, OAS1/2/3, RNase L, PKR), were transfected in vitro to assess IFN-I and proinflammatory cytokine secretion, global translation (puromycin readouts), and tumor cell death. Lead 3p-RNA candidates were evaluated in vivo in a subcutaneous MC38 tumor model in C57BL/6 mice to measure tumor growth control and systemic T-cell responses.

Results Our findings revealed clear 3p-RNA-mediated length-dependent effects on cytokine and cell death induction in both human and murine models. IFN-I production increased with RNA length until a maximum was reached, then declined at longer lengths coincident with enhanced activation of OAS/RNase L and PKR-mediated translational arrest. In contrast, tumor cell death steadily increased with RNA length. Kinetic analysis identified PKR as the main driver mediating rapid translation inhibition after stimulation with long 3p-RNA thereby negatively regulating IFN-I output. In vivo, longer 3p-RNA improved tumor control in the MC38 model and was associated with stronger tumor specific T cell responses.

Conclusion Tuning 3p-RNA length differentially engages RIG-I, OAS/RNase L, and PKR to shift the balance between cytokine production and tumor cell death. While long 3p-RNA suppresses IFN-I via early translation arrest, it augments tumor cell death and likely increases antigen availability, supporting the development of length-optimized 3p-RNA as innate agonists to potentiate adaptive antitumor immunity.

P01.03 **ABSTRACT WITHDRAWN**

Poster Session 2. Cellular Therapies (Including Combinations)

P02.01 FROM GENOMIC BREAKPOINTS TO TCRS: A HIGH-RESOLUTION PIPELINE FOR IDENTIFYING FUSION-DERIVED NEOANTIGENS IN HEMATOLOGICAL MALIGNANCIES

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10.1136/JITC-2026-ITOC.15

Background Tumor-specific gene fusions generate neoantigens that can be recognized by T cells, providing potential targets for personalized immunotherapy. High-resolution profiling of T cell receptor (TCR) repertoires using next-generation sequencing (NGS) enables the identification of antigen-specific TCRs for the development of adoptive T cell therapy.

Methods 1,189 candidate fusion sequences were first inferred from RNA sequencing data of hematological malignancy patients, primarily those with acute leukemias (ALs). Computational protein reconstruction was performed by building an informatics pipeline capable of predicting the fusion peptide from the two genes involved and the breakpoint indicated in the database. Prioritization for immunogenicity was performed using Immune Epitope Database (IEDB) prediction tools, integrating MHC binding affinity prediction, antigen processing pathway analysis, and immunogenicity assessment. Selected fusion-derived peptides were synthesized and used to stimulate peripheral blood mononuclear cells from healthy donors. After peptide-pulsing through HLA-matched antigen-presenting cells, expanded antigen-specific T cells were phenotypically characterized and subjected to single-cell TCR sequencing. Identified TCRs were cloned, expressed as mRNA, and functionally evaluated in an RNA-based Jurkat T cell model.

Results By applying our bioinformatic pipeline, we derived 143 putative FASTA files, from which we chose and tested two HLA-A02-restricted epitopes, along with control epitopes from the literature.[1 2] A negative condition (no epitope) was included. Our results showed successful in vitro expansion of antigen-specific T cells and isolation of TCRs recognizing fusion-derived neoantigens. Preliminary in vitro validation indicates correct expression of fusion-reactive TCRs, with specificity demonstrated so far in one candidate, supporting proof-of-concept for TCR-based targeting of fusion peptides originating from genomic translocation events in leukemia, with planned evaluation in primary T cells for anti-tumor activity.

Conclusion Fusion-derived neoantigens represent a novel class of targets for personalised T cell therapy. This study demonstrates the feasibility of using NGS-based TCR profiling to identify and characterize tumor-specific TCRs, laying the foundation for future adoptive T cell therapies in genomic fusion-driven malignancies like leukemias.

Support This research is supported by EU - Next GenEU - NRRP M6C2 - PNRR-POC-2022-12375862 FUSION-TARGET - CUP: E43C22001080006

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P02.02 IDENTIFICATION OF TUMOR MICROENVIORNMENT-DEPENDENT RESISTANCE AND EMERGENCE OF PERSISTER CELLS TO KRAS INHIBITION

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10.1136/JITC-2026-ITOC.16

Background Non-small cell lung cancer (NSCLC) remains a major cause of cancer-related deaths worldwide and is often

driven by mutations in the KRAS oncogene. Interestingly, smokers most often bear KRASG12C mutations, whereas KRASG12D mutations are more common in non-smokers. KRASG12C inhibitors were FDA-approved in 2021 and have revolutionized personalized therapeutic approaches for lung cancer. However, resistance is widespread, comprising genetic alterations in RAS, gene amplification, and compensatory pathway activation, along with changes in the tumor microenvironment (TME). For KRASG12D inhibitors now emerging for clinical testing, therapeutic resistance is also predicted.

Methods To understand both tumor microenvironmental changes induced by oncogenic KRAS and TME-dependent resistance mechanisms, we developed a new orthotopic model of KRASG12D-driven lung cancer, derived from our published genetically engineered mouse model of KRASG12D NSCLC (Lasse-Opsahl and Barravecchia et al., JCI Insight 2025). Single-cell RNA sequencing data from lung tumor tissue of mice in which KRAS was inhibited, either genetically or pharmacologically with the pan-RAS inhibitor RMC-7977, demonstrated major changes in several cell clusters.

Results Not surprisingly, both genetic and therapeutic inhibition of oncogenic KRAS reduced proliferating tumor cells. In fact, 95% of proliferating cells were lost in lungs where KRASG12D expression was turned 'OFF' and 90% were lost in RMC-7977-treated mice. Gene set enrichment analysis (GSEA) of the proliferating tumor cell cluster confirmed strong oncogenic KRAS signaling activity in these cells and thus, dependency on oncogenic KRAS. Despite loss of most proliferating tumor cells, further sub-clustering revealed that a small subset of tumor cells persisted. These 'persister' cells displayed a low GSEA 'hallmark oncogenic KRAS signaling' signature in 'ON' tumors, and thereby likely allowed evasion of KRAS-targeted elimination. However, upon KRAS inhibition, persister cells were activated and exhibited dramatic upregulation of the GSEA hallmark KRAS signaling pathway signature (39% in OFF and 16% in RMC-7977-treated tumors, respectively). Furthermore, persister cells expressed multiple RAS/MAPK signaling genes, including Raf and Cdc42, likely contributing to evasion and resistance to KRAS inhibition.

Genetic and pharmacological inhibition of oncogenic KRAS eliminated not only tumor cells, but also IFN-responsive $CD8^+$ cytotoxic T-cells. Loss of these CTLs may have been caused by reduced antigen presentation and inflammatory cues associated with the dramatic loss of tumor cells observed upon KRAS inhibition. In contrast, KRAS inhibition drove a marked expansion of $CTLA4^+$ $CD4^+$ T cells, indicating adaptive resistance in the TME to KRAS inhibition.

Conclusion In conclusion, our findings reveal major changes in both tumor and T-cell composition following KRASG12D inhibition and potential TME-dependent mechanisms of resistance to KRAS inhibitors.

P02.03 TARGETING CDCP1 WITH SINGLE-DOMAIN ANTIBODY-BASED CAR T CELLS TO TREAT SOLID TUMORS

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10.1136/JITC-2026-ITOC.17

Although CAR T cell therapy has shown profound success for the treatment of hematological diseases, it still poses a challenge for solid tumors. Prostate cancer is the 4th most common cancer worldwide and the most common cancer among men. Advanced and highly metastatic stages show high expression levels of the cell surface glycoprotein CDCP1 (CD318), which is associated with poor prognosis.

After a successful immunization campaign and characterization of binding specificity, epitopes, and affinity, five different anti-CDCP1 single-domain antibodies (sdAbs) were selected and used as antigen-binding moieties in our second-generation Nano-CAR T cells. Through co-culture and real time killing assays we initially investigated the activation and killing potential of our Nano-CARs. Furthermore, we assessed the efficacy in vivo by establishing an adoptive Nano-CAR transfer protocol using immunocompetent C57BL/6J mice bearing human CDCP1 overexpressing MC38 mouse colon carcinoma cells.

All Nano-CARs showed a specific and profound induction of TNF-α and IFN-γ when exposed to CDCP1 expressing cells in vitro; either in an overexpression system or at endogenous levels. Furthermore, all of our Nano-CARs showed a rapid and efficient killing of target cells in real-time killing assays. Upon adoptive transfer of CDCP1-Nano-CARs into tumor-bearing mice, we were able to observe a tumor growth inhibition resulting in significantly prolonged survival of treated mice. Due to our syngeneic mouse model, we were also able to investigate potential adverse immune related events mediated by on-target off tumor activity.

Overall, we provide first evidence in vitro and in vivo that CDCP1-Nano-CAR T cells could be developed into a novel promising cellular therapy for solid tumors.

P02.04 COMPOSITION OF PERIPHERAL BLOOD IMMUNE-CELLS IN PATIENTS WITH LEUKEMIA AFTER ALLOGENEIC STEM-CELL-TRANSPLANTATION(HSCT) IN PATIENTS WITH VS. WITHOUT CHRONIC(C) OR ACUTE(A) GRAFT-VERSUS-HOST-DISEASE(GVHD) AND HEALTHY DONORS

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10.1136/JITC-2026-ITOC.18

Background aGvHD/cGvHD are common and potentially life-threatening autoimmune complications after HSCT, mediated by immune cells. Treatment aims to suppress excessive immune activity while maintaining graft versus leukemia effects. The impact of treatment on the provision of inflammatory or inhibitory immune cell subsets is poorly understood.We compared myeloid, adaptive and innate immune cell compositions in aGvHD/cGvHD vs non-GvHD HSCT patients or healthy controls before treatment (baseline) and correlated findings with clinical patients' characteristics. Finally, we deduced knowledge about immune cells involved in patients after HSCT that did not develop GvHD ('NoGvHD') vs GvHD patients.

Methods Flow cytometric cell analyses of blood samples (H=10, NoGvHD=19, cGvHD=18, aGvHD=6) were observed at baseline. Statistical analysis was performed.

Results

Cells in cGvHD compared to NoGvHD

- Myeloid: ↓ DCtol/MDSCs, ↑ myeloid dysfunction
- Adaptive: ↑ effector-memory, ↓ regulatory/anti-tumor subsets
- Innate: ↑ differentiated/exhausted NK/CIK

Cells in aGvHD compared to NoGvHD

- Myeloid: ↓ inhibitory monocytes/DCtol
- Adaptive: ↑ strong late T-cell proliferation, ↓ Tregs/B cells
- Innate: ↓ NK56bri, ↑ destabilized/exhausted NK/iNKT

Cells in aGvHD compared to cGvHD

- Myeloid: acute collapse (aGvHD) vs. chronic dysfunction (cGvHD)
- Adaptive: extreme effector proliferation (aGvHD) vs. persistent activation (cGvHD)
- Innate: loss of early NK regulators (aGvHD) vs. NK56dim dominance (cGvHD)

Cells in NoGvHD compared to GvHD development

- Myeloid: ↑ tolerogenic DC/MDSC
- Adaptive: ↑ T/Treg, ↓ activation/proliferation
- Innate: ↓ NK/CIK exhaustion

Cells in skin aGvHD compared to no-skin aGvHD

- Myeloid: ↓ tolerogenic signatures
- Adaptive: ↑ activation/homing markers, ↓ regulatory subsets
- Innate: ↑ NK/CIK exhaustion, ↑ TH1/TH17 skewing

Cells in overlap cGvHD compared to non-overlap cGvHD

- Myeloid: ↓ tolerogenic myeloid cells
- Adaptive: ↑ PD-L1/TIGIT, ↓ Tregs
- Innate: ↑ NK/CIK exhaustion

Cells in severe cGvHD compared to mild cGvHD

- Myeloid: ↑ activated/CTLA-4^{+} MDSC/DC
- Adaptive: ↑ proliferating/gut-homing T cells, ↓ Th1/Treg
- Innate: ↑ NK/CIK exhaustion

Cells in multi organ cGvHD compared to single organ cGvHD

- Myeloid: ↑ inflammatory monocytes/MDSCs
- Adaptive: ↑ γδ^{+}/Intβ7^{+} activation, ↓ Tregs
- Innate: ↑ checkpoint-positive NK/CIK, ↓ NK56bri

Cells in DLI-exposed compared to non-DLI exposed cGvHD

- Myeloid: ↓ tolerogenic myeloid function
- Adaptive: ↑ activation/gut-homing, ↓ Tregs
- Innate: ↑ NK/CIK exhaustion

Conclusion Post-HSCT, NoGvHD patients who did not develop GVHD maintain regulatory myeloid and adaptive features with limited T/NK activation, whereas GvHD patients, with especially skin aGvHD, severe cGvHD, multi-organ cGvHD, or DLI-treated cGvHD, showed myeloid suppression, T-cell hyperactivation, and NK/CIK exhaustion, reflecting distinct inflammatory and tissue-homing immune signatures.

This work was funded by an external collaborative research (ECR) grant provided by Therakos EMEA Limited.

P02.05 ADAPTIVE AND MYELOID DYNAMICS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN ACUTE OR CHRONIC GRAFT-VERSUS-HOST DISEASE UNDERGOING EXTRACORPOREAL PHOTOPHERESIS VERSUS NON–GRAFT-VERSUS-HOST DISEASE AND HEALTHY DONORS

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10.1136/JITC-2026-ITOC.19

Background Allogeneic stem cell transplantation (HSCT) is often associated with Graft-versus-Host Disease (GvHD) resulting from dysregulated alloreactivity. Extracorporeal photopheresis (ECP) is an established therapy for immunomodulation in GvHD, but underlying cellular mechanisms remain largely unclear. We compared myeloid and adaptive immune cell compositions from patients with acute GvHD (aGvHD) / chronic GvHD (cGvHD) receiving ECP to healthy individuals and patients without GvHD after HSCT (NoGvHD).

Methods ECP was administered using the TherakosTM CELLEXTM Photophoresis system. Flow cytometric analysis of blood samples (Healthy N=10, NoGvHD N=19, cGvHD N=18, aGvHD N=6) was performed at baseline (d0) and after 13 weeks (d90), with or without ECP treatment, followed by statistical analyses.

Results

Baseline (d0): cGvHD vs. NoGvHD

- Adaptive: ↑ memory T-cells, ↓ Tregs, ↓ Bregs
- Myeloid: ↑ activated dendritic cells (DCs), ↓ myeloid derived suppressive cells (MDSCs)

NoGvHD (Course d0 → d90)

- Adaptive: T-cells, Tregs, Bregs → stable
- Myeloid: Monocytes, DCs, MDSCs → stable

cGvHD during ECP (Course d0 → d90)

- Adaptive: memory T-cells ↓ (composition similar to NoGvHD), T-cell proliferation ↓, Tregs ↑ (partial recovery), Bregs ↑ (partial recovery)
- Myeloid: activated DCs ↓, inflammatory monocytes ↓, MDSCs ↑ (composition similar to NoGvHD)

Post-treatment (d90): cGvHD vs. NoGvHD

- Adaptive: memory T-cells still ↑, but lower compared to d0, Tregs still ↓ despite partial recovery
- Myeloid: activated DCs stay ↑, MDSCs ↓

Conclusions At baseline compared to NoGvHD, cGvHD before ECP showed pronounced adaptive and myeloid dys-

regulation. By d90, NoGvHD profiles showed stable levels of cells while ECP induced partial normalization of dysregulated immune compartments in cGvHD (compared to d0). Immune hyperactivation persisted but was attenuated after ECP. Clinical correlations will be presented at the congress.

This work was funded by an external collaborative research (ECR) grant provided by Therakos EMEA Limited.

P02.06 RELIABLE IFN-γ QUANTIFICATION FOR T CELL FUNCTIONAL ANALYSIS: ANALYTICAL PERFORMANCE EVALUATION OF A CYTOKINE BEAD ASSAY

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10.1136/JITC-2026-ITOC.20

Background T cell therapies, including CAR T cells, are rapidly expanding across therapeutic areas, from oncology, to more recently, autoimmune disease. This expanding clinical scope increases the demand for analytical methods that reliably characterize their functional properties across development stages, from early research through process optimization and validated assays. IFN-γ secretion is a widely used as a readout of T cell activation and potency. However, consistent quantification requires rigorous evaluation of assay accuracy, precision, linearity, and specificity. Here, we describe an analytical performance evaluation of a MACSPlex® assay, conducted in accordance with current regulatory guidance, to assess its suitability for reproducible IFN-γ quantification.

Methods IFN-γ quantification performance was evaluated using a MACSPlex® assay in alignment with regulatory expectations for ligand-binding assay characterization. Accuracy, precision, and linearity were assessed using recombinant IFN-γ spike-and-recovery samples across eight nominal concentrations (0-100.000 pg/mL). Dilutional integrity was evaluated by spiking samples at 150.000 pg/mL IFN-γ and quantifying serial dilutions. Matrix effects were assessed by preparing matched spike concentration in two different diluents and comparing resulting accuracy and precision. All samples were acquired on a MACSQuant® Analyzer, and quantified using ExpressMode® automated analysis.

Results The assay demonstrated strong analytical performance across key parameters. Accuracy and precision consistently met predefined acceptance criteria between 32 and 100.000 pg/mL, with inter-assay precision and overall accuracy well within ±20%. Linearity was maintained across the calibration range, with log-log regression yielding R2=0.9918, indicating proportional response over more than 4 orders of magnitude. Dilutional integrity testing showed that all evaluated dilution levels, up to 1:4096, remained within accuracy and precision acceptance limits, demonstrating that samples exceeding the upper limit of quantification (ULOQ) can be reliably quantified following appropriate dilution. Matrix comparison studies revealed buffer-dependent assay performance: the MACSPlex® Buffer supported accurate and precise quantification across the validated range, whereas an alternative diluent resulted in deviations outside acceptance criteria.

Conclusion This study provides a comprehensive analytical performance evaluation of IFN-γ quantification using a MACSPlex® assay. The assay demonstrated robust accuracy, precision, linearity, and dilutional integrity over a broad dynamic range, and reliably quantified IFN-γ across multiple sample types when used under validated matrix conditions. These findings establish a strong analytical foundation for applying this assay to T cell functional characterization, supporting exploratory studies through standardized potency and immunomonitoring workflows.

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P02.07 IMPACT OF HUMAN AB SERUM ON CAR T CELL MANUFACTURING AND METABOLIC PROPERTIES OF THE DRUG PRODUCT

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10.1136/JITC-2026-ITOC.21

Background In recent years, Chimeric antigen receptor (CAR) T cells emerged as a new and successful therapeutic approach. While CAR T cells display high efficacy in late cancer stages of liquid tumors and autoimmune diseases, they still face challenges associated with incoming process materials. There is an interest in reducing the usage of human AB (hAB) serum during manufacturing and understanding the accompanying impact on CAR T cell characteristics. Therefore, we characterized the impact of reduced hAB serum supplementation on CAR T cell properties in freshly formulated drug products (DP).

Materials and Methods Using the CliniMACS Prodigy® instrument, CAR T cells were manufactured in a 12-day standard process in which the culture medium was supplemented with 3% human AB serum for either the initial 5 or full 12 days. At day 12, CAR T cells were harvested and fresh DPs formulated in clinically relevant doses in CliniMACS Formulation Solution®.

Additionally, samples were frozen for single cell (sc)-RNA sequencing (n = 3). Samples were thawed and processed in batches using the 10xGenomics workflow. Sequencing was conducted on the Illumina NextSeq 2000 platform. Data analysis was done using 10x Genomics software, cellranger and the Seurat pipeline in R. Enrichment analysis was performed using clusterProfiler and the Reactome database. The metabolic state of fresh DP was assessed via flow cytometry either by quantifying translational rate according to the SCENITH protocol[1] or staining for mitochondrial mass.

Results The serum conditions resulted in comparable drug product characteristics such as cellular composition, transduction efficacy and T cell differentiation.

Sc-sequencing confirmed comparable cellular composition based on UMAP plots. Nevertheless, comparing both DP serum conditions resulted in 126 differentially expressed genes (DEG). Gene Ontology (GO) enrichment was used to interpret the resulting gene list. As a result, genes related to translational and energy metabolism were found to be differentially expressed. Next, we investigated those two categories on a functional level using a flow cytometry-based approach. Surprisingly, neither the SCENITH assay nor staining of the mitochondrial mass displayed significant metabolic changes of DPs manufactured with different serum conditions.

Conclusion hAB serum is a highly complex component that provides various nutrient sources during CAR T cell manufacturing. While reducing its availability results in comparable culture properties, sc-sequencing identified DEG related to metabolism. However, the SCENITH protocol and mitochondrial mass did not support changes in translational and energy metabolism. Yet, other metabolism pathways remain to be tested to better understand the effect of hAB serum.

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All authors are employees of Miltenyi Biotec B.V. & Co. KG.

P02.08 PRECISE AND FLEXIBLE FORMULATION AND FILLING OF CAR T CELLS IN A FULLY CLOSED AND AUTOMATED SYSTEM - THE NEW CLINIMACS PRODIGY TCTF PROCESS

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10.1136/JITC-2026-ITOC.22

Background Chimeric Antigen Receptor (CAR) T-cell therapies have resulted in durable cures in patients suffering from hematological cancers and autoimmune diseases. An increasing spectrum of disease indications, a rising number of eligible patients, and commercialization of CAR-T therapy necessitate a robust, reliable, and scalable manufacturing platform for this highly personalized medicine. We present the T cell Transduction and Automated Formulation (TCTf) process as a comprehensive, closed, and automated platform for CAR-T cell manufacturing. This advanced methodology enhances the current CliniMACS Prodigy® T cell Transduction (TCT) process by increasing flexibility through an innovative formulation and filling technique. It improves scalability and reduces contamination risks by minimizing open processing steps. The new, free-of-charge and off the shelf TCTf application is facilitating process initiation with fresh or cryopreserved leukapheresis and allows the choice of flexible cell dose and final filling parameters of T cell products either for direct infusion or cryopreservation.
Methods Leukapheresis from healthy donors (N=5) was processed using the novel TCTf process. CD4+ and CD8+ T cells were enriched, activated with TransAct® and transduced with a lentiviral vector. Cellular expansion and transduction efficiency was evaluated at different timepoints. CliniMACS® Formulation Solution was used for fresh product filling. Options for cryopreservation of final drug product were assessed using Formulation solution and either the 3x CliniMACS Cryosupplement or a comparable 2x DMSO-based cryobuffer. Filling-accuracy was determined by measuring different product bags with varying volumes using a scale, and cell concentration was measured using the MACSQuant analyzer. CAR-T cell functionality was evaluated through cytotoxicity assays and cytokine secretion profiles via MACSPlex for fresh filled and cryo-recovered CAR-T cells.
Results CAR-T cells manufactured using the TCTf process showed robust in-vitro anti-tumor cytotoxicity and a potent secretion of TNF-a and IFN-g, while no cytotoxicity against antigen-negative target cells was observed. T cells were 25.7%±8.7 of central memory and 57.6%±11.6 of stem-cell-like phenotype that is associated with clinical efficacy. The cryopreserved products maintained a viability of 88.4%±1.3 up to three hours after thawing and storage at room temperature. The introduction of a pump specific adjustment factor determination in the TCTf software, in combination with the new all-in-one Tubing Set 521 and the CliniMACS® Formulation Unit, enables precise volume transfers (in this study deviating from -1,70% to 2,24%) and final filling of exact cell doses in differently sized product bags.
Conclusion The TCTf process enhanced comprehensive CAR-T cell manufacturing through a fully closed and automated system, eliminating open step operator interactions. The system allows flexible filling of up to 310 mL drug Product, across eight different product bag sizes, ranging from 10 to 300 mL. This facilitates the formulation at a specific concentration, ranging from 0.5 to 150x 10^6 cells/mL, suitable for either direct infusion or cryopreservation.

P02.09 CSF1R$^+$ MYELOID CELLS MEDIATE RESISTANCE TO CAR-T-CELL THERAPY IN LUNG CANCER BRAIN METASTASES

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10.1136/JITC-2026-ITOC.23

Background Immunotherapies in the treatment of brain metastases remain a major challenge largely due to the highly immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages and microglia (TAM/M) have emerged as key mediators of immune suppression. Although EpCAM-directed CAR T-cell therapy has shown safety and efficacy in preclinical models, limited intratumoral persistence contributes to tumor recurrence. However, whether modulation of TAM/M can enhance CAR T-cell infiltration and efficacy remains unclear.
Methods We utilized our established syngeneic orthotopic cerebral metastasis mouse model, combining a chronic cranial window with repeated intracerebral two-photon laser scanning microscopy to enable single-cell resolution imaging of fluorescent EpCAM/tdTCAR T-cells, TAM/M, and lewis lung carcnioma cells over weeks. EpCAM/tdTCAR T-cells were stereotactically injected adjacent to the tumor site. TAM/M depletion was achieved via CSF1R inhibition using a PLX 3397 diet. To further characterize the spatial organization of cellular and humoral components within the TME, immunofluorescence staining was performed.
Results Immunohistochemistry revealed a high intratumoral density of TAM/M. CSF1R inhibition significantly reduced TAM/M numbers, resulting in higher intratumoral CAR T-cell densities compared to control diet. Increased CAR T-cell infiltration correlated with reduced tumor growth and improved

survival. In vivo imaging demonstrated that TAM/M interact with CAR T-cells, which were reduced following CSF1R inhibition, suggesting TAM/M-mediated impairment of CAR T-cell trafficking.

Conclusion Our findings demonstrate that TAM/M contribute significantly to CAR T-cell suppression in lung cancer brain metastases. Targeting CSF1R on TAM/M improved CAR T-cell trafficking, intratumoral persistence and therapeutic efficacy resulting in a survival benefit. These results pave the way for combinatorial strategies incorporating CSF1R inhibition to further enhance CAR T-cell functionality and improve outcomes in the treatment of brain metastases.

P02.10 DEVELOPMENT OF NECTIN-2 SPECIFIC NANO-CARS FOR THE TREATMENT OF MELANOMA

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10.1136/JITC-2026-ITOC.24

Background Immune checkpoint blockade has revolutionized the treatment of melanoma. However, a significant proportion of patients fail to benefit. Recently, the first adoptive T-cell therapy for melanoma received regulatory approval, marking a major milestone for the field and paving the way for the development of next-generation cellular immunotherapies. Here we set out to develop a CAR T cell therapy for the treatment of melanoma using Nectin-2 specific single domain antibodies (sdAb) as antigen recognition domain.

Methods First, we conducted an immunisation campaign, producing and selecting a diverse pool of 14 sdAbs using phage display and ELISA. Flow cytometric characterisation revealed 10 sdAbs specific for human Nectin-2 and three with cross-reactivity to murine Nectin-2. Importantly, none of the sdAbs bound to other Nectin family members, confirming high target specificity. A variety of Nectin-2 sdAbs were cloned into our second-generation CAR construct, enabling efficient transduction of human or mouse T cells for the generation of Nectin-2 Nano-CARs.

Results Upon co-culture of Nectin-2 Nano-CARs with human or mouse melanoma cell lines, we observed robust IFN-γ and TNF-α production. Real-time killing assays showed efficient and dose-depended melanoma cell killing by our Nano-CARs, while Nectin-2 knockout cells were largely resistant. Building on these results, we established an in vivo CAR-transfer model in immunocompetent C57Bl/6 mice, demonstrating disease control with our Nectin-2 Nano-CARs in the syngeneic B16 melanoma model. This model enabled us to assess both the efficacy and the safety profile of our Nano-CARs.

Conclusion Next, we will investigate the long-term efficacy of Nano-CARs in vivo and conduct further in-depth characterisation to identify the optimal sdAb. Taken together, we here provide first evidences that targeting Nectin-2 with Nano-CAR T cells represents a promising treatment approach for melanoma.

P02.11 IMPACT OF SYSTEMIC POLYCHEMOTHERAPY IN PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA ON CAR T CELL PRODUCTION AND FUNCTION

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10.1136/JITC-2026-ITOC.25

Background Pancreatic ductal adenocarcinoma (PDAC) remains a major clinical challenge, characterized by late diagnosis, limited therapeutic options and poor prognosis. With only a small percentage of patients qualifying for surgical removal, systemic treatment with chemotherapy continues to represent the backbone of PDAC therapy. In recent years, chimeric antigen receptor (CAR) T cell therapy has reshaped the treatment landscape of cancer and fueled high expectations as a potential therapeutic option in difficult-to-control diseases such as PDAC. Its efficacy however remains variable and predominantly restricted to hematological malignancies with clinical trials of CAR T cell therapy in solid tumors mostly falling short of expectations. One potential pitfall of CAR T functionality in this setting may be concomitant polychemotherapy (CTX), which remains standard-of-care in PDAC patients and will have been applied when CAR T generation takes place. Therefore, a better understanding of the implications of different CTX regimen on T cell functionality is needed to guide potential combinations of CAR T cell therapy and CTX in PDAC.

Methods We generated patient-derived, 2nd generation anti-Mesothelin (MSLN) directed CAR T cells from 30 PDAC patients subjected to one of five CTX regimens (Gemcitabine, Gemcitabine/Nab-paclitaxel, Gemcitabine/Nab-paclitaxel/Azacitidine, FOLFOX or FOLFIRINOX) at our center after informed consent. In vitro analysis of CAR T products was performed at three distinct timepoints: before CTX (TP1), after first (TP2) and after multiple cycles of CTX (TP3). The analysis included phenotypic profiling of T cells and assessment of successful CAR T cell generation and expansion by flow cytometry. Additionally, CAR T anti-tumor capacity was assessed via cytokine release assays and luciferase and xCELLigence-based cytotoxicity readouts after co-culture with a PDAC cell line. Healthy donor (HD) samples served as assay validations.

Results We could demonstrate successful generation of anti-MSLN CAR T cells from patient peripheral blood mononuclear cells (PBMC) with adequate yield and CAR transduction rate. While overall CTX exposure resulted in decreased T cell counts, no relevant impairment of T cell viability was noted. Baseline phenotype of isolated T cells did not notably change across timepoints and CTX regimen, except for a slight reduction in CD8+ memory subset formation. T cells of patients receiving Gemcitabine/Nab-Paclitaxel regimen showed lower

CAR transduction efficacy and expansion rate as compared to T cells of patients receiving FOLFOX and FOLFIRINOX based therapy, indicating an impact on T cell fitness through Gemcitabine/Nab-Paclitaxel regimen.

Conclusion Overall, our findings show that CAR T cells can effectively be generated from PDAC patients with prior or concomitant CTX treatment, though certain regimen may impact cellular fitness and expansion potential of CAR T products. These result, while they may vary between entities, underscore the necessity for further investigations optimizing combinations of CAR T cell application and chemotherapy in solid tumors.

P02.12 IDENTIFICATION OF AN HLA-A*11:01-RESTRICTED KRASG12V-SPECIFIC T CELL RECEPTOR WITH STRONG ANTITUMOR RESPONSES IN PATIENT-DERIVED CANCER MODELS

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10.1136/JITC-2026-ITOC.26

Background KRAS is the most frequently mutated oncogene in human cancers, with codon 12 mutations (especially G12V) highly prevalent in pancreatic, lung, and colorectal cancers, positioning KRAS neoantigens as attractive targets for adoptive T cell therapy. Previous studies have demonstrated that peptides encoded by this mutation are abundantly presented on HLA-A*11:01 and less abundantly on HLA-A*03:01.[1]

Materials and Methods We established an in vitro co-culture pipeline utilizing naïve CD8+ T cells from healthy donors to identify and isolate T cell receptors (TCRs) specific for KRAS mutant peptides. Candidate TCR was identified, sequenced, and engineered into primary donor T cells via retroviral transduction. Functional assays were performed using tumor cell lines and patient-derived organoids.

Results Using this approach, we identified a novel TCR that recognizes the KRASG12V neoantigen presented by HLA-A11:01. TCR-engineered T cells exhibited high antigen-specificity and sensitivity, robustly recognizing and eliminating HLA-A11:01-positive tumor cells expressing KRASG12V, with negligible cross-reactivity against 23 KRAS wild-type cell lines derived from diverse cancer types. Moreover, TCR-transduced T cells demonstrated potent antitumor activity against KRASG12V expressing patient-derived colorectal cancer organoids.

Conclusion These results underscore the therapeutic promise of HLA-A*11:01-restricted TCR-based immunotherapy for KRASG12V-driven malignancies, particularly in populations where this HLA allele is prevalent.

REFERENCES

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P02.13 COMBINATION OF ADOPTIVE CELL THERAPY WITH ANV419, A SELECTIVE IL-2Rβ/γ AGONIST, IN PATIENTS WITH ADVANCED MELANOMA

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10.1136/JITC-2026-ITOC.27

Background Adoptive cell therapy (ACT) using ex vivo-expanded tumor-infiltrating lymphocytes (TILs) has demonstrated substantial clinical benefits for patients with treatment-resistant metastatic melanoma. Standard TIL-ACT involves the administration of high-dose interleukin-2 (HD IL-2) to support the expansion, persistence and function of the transferred TILs. However, this regimen is associated with considerable systemic toxicity, which limits patient eligibility and often requires intensive care unit monitoring and interventions. Furthermore, HD IL-2 can expand regulatory T cells (Tregs) by signaling through the high-affinity IL-2 receptor α chain, potentially constraining antitumor immunity.

Methods BaseTIL-03M (NCT05869539) is a phase I clinical trial evaluating the safety and preliminary efficacy of TIL-ACT in combination with ANV419, an IL-2Rβ/γ-selective IL-2 variant with an extended half-life and preferential stimulation of effector lymphocytes. Ten patients with metastatic cutaneous melanoma refractory to standard therapies were enrolled and received TIL-ACT followed by two intravenous doses of ANV419 (243 mcg/kg), administered on the day of TIL transfer and again on day 14. Assessment of clinical safety and efficacy was complemented by comprehensive translational analyses, including evaluation of systemic immune activation by multiplex serum cytokine profiling seven days after TIL transfer. These data were compared with TIL-ACT cohorts from other clinical trials receiving low-dose (LD) IL-2 (125'000 IU/kg once daily for 10 days) or HD IL-2 (600'000 IU/kg every 8 hours for up to 8 doses).

Results As reported previously at the ESMO Immuno-Oncology Congress 2025, TIL-ACT in combination with ANV419 was well tolerated, with no dose-limiting toxicities and a 30-day mortality rate of 0%. Multiplex serum profiling revealed a distinct immune activation signature following TIL transfer with ANV419, characterized by increased levels of granzyme A, granzyme B, TNF-α, and soluble CD137. This cytokine profile differed from that observed in TIL-ACT cohorts receiving LD or HD IL-2, suggesting qualitatively distinct immune modulation by β/γ-selective IL-2 signaling. Comprehensive analyses of intratumoral immune composition by multiplex spatial proteomics, together with assessments of T cell differentiation states and clonal dynamics by flow cytometry and TCR sequencing, are ongoing.

Conclusion While clinical data indicate that combining TIL-ACT with ANV419 is feasible and potentially better tolerated than conventional HD IL-2, translational data provide evidence for distinct immunological effects of ANV419 compared with LD or HD IL-2 regimens. Together, these findings support the further investigation of IL-2Rβ/γ-selective IL-2 variants as a strategy to enhance the therapeutic index of TIL therapy.

P02.14 EFFECTS OF EXTRACORPOREAL PHOTOPHERESIS (ECP) ON LEUKEMIA-SPECIFIC AND ANTI-LEUKEMIC IMMUNE CELL SUBSETS IN LEUKEMIA PATIENTS AFTER STEM CELL TRANSPLANTATION (HSCT) WITH ACUTE OR CHRONIC GRAFT-VERSUS-HOST-DISEASE

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10.1136/JITC-2026-ITOC.28

Background Acute and chronic GvHD are major complications after HSCT, and ECP is an established treatment, though its effects on leukemia-specific immunity remain unclear. We analyzed leukemia-specific and antitumor-associated T cell subsets in patients with acute GvHD, chronic GvHD, no GvHD, and healthy donors at baseline and after 3 months of ECP to identify GvHD-, ECP-, and relapse-associated immune signatures.

Methods ECP was performed using the TherakosTM CELLEXTM system. Flow cytometric analyses were performed on patient blood samples and d90 (after ECP), focusing on leukemia-specific (DEG+/INC+), antitumor directed and Thelper cells. Statistical analyses were conducted

Results

noGvHD d0 vs Healthy controls:

- TH cells H vs. noGvHD: TH17↑, TH1↑, TH2↑
- Integrinß7+T4-↓ and γδ+ T4+↓ in H

noGvHD d0 vs. d90:

- Degranulating cells: CIK cells ↓, NK cells ↓, Tem cells ↓
- INFγ producing cells: CIK↓, NK↑
- TH cells: TH17↑, TH1 and TH2 stable

cGvHD d0 vs. d90 under ECP:

- Degranulating cells: CIK cells ↓, NK cells↑, Breg↑, Tem↓, Tcm↑
- INFγ producing cells: CIK cells ↓, NK cells ↓, Tcm↑
- TH cells: TH17 stable, TH1↑, TH2↓

aGvHD d0 vs. d90 under ECP:

- Degranulating cells: CIK cells ↓, NK cells ↓
- INFγ producing cells: CIK cells↑, NK cells↑, Tem cells↑
- TH cells: TH17, TH1 and TH2 stable

cGvHD vs. noGvHD d0:

- Degranulating cells cGvHD vs. noGvHD: CIK cells↓, NK cells↓, Tcm↓, Tem↓, Breg↑
- INFγ producing cells cGvHD vs. noGvHD: CIK cells↓, NK cells↓, Tcm↓, Tem↑
- TH cells cGvHD vs. noGvHD: TH17↓, TH1↓, TH2↓

aGVHD vs. noGvHD d0:

- Degranulating cells aGvHD vs. noGvHD: CIK cells↑, NK cells↑, Breg ↑, Tcm↓, Tem↓
- INFγ producing cells aGvHD vs. noGvHD: CIK cells↓, NK cells↓, Tcm↓, Tem↓
- TH cells aGvHD vs. noGvHD: TH17↓, TH1↓, TH2↓

cGvHD under ECP vs. noGvHD d90:

- Degranulating cells cGvHD vs. noGvHD: CIK cells↑, NK cells↑, Breg↑, Tcm↓, Tem↓
- INFγ producing cells cGvHD vs. noGvHD: CIK cells↑, NK cells↑, Tcm↑, Tem↓
- TH cells cGvHD vs. noGvHD: TH17↓, TH1↑, TH2↓

aGvHD under ECP vs. noGvHd d90:

- Degranulating cells aGvHD vs. noGvHD: CIK cells↑, NK cells↑, Tem↑
- INFγ producing cells aGvHD vs. noGvHD: CIK cells↑, NK cells↑, Tcm↓, Tem↑
- TH cells aGvHD vs. noGvHD: TH17↓, TH1↑, TH2↓

Summary At d0, both aGvHD and cGvHD patients showed reduced degranulating and IFNγ-producing CIK, NK, and Tcm cells, as well as lower TH17, TH1, and TH2 levels compared with noGvHD. Both GvHD groups also had decreased DEG/IFNγ$^+$ Tem and increased DEG-Breg cells. Intβ7$^+$T4$^-$ and γδ$^+$T4$^+$ subsets were lowest in healthy donors.

At d90, aGvHD and cGvHD patients showed higher degranulating and IFNγ-producing CIK andNK cells than noGvHD, along with reduced TH17/TH2 and increased TH1 levels. cGvHD patients additionally showed reduced DEG-Tcm and increased DEG-Breg cells.

Overall, noGvHD patients showed stable, GVHD patients under ECP variable immune subset compositions.

Funding This work was funded by an external collaborative research (ECR) grant provided by Therakos EMEA Limited

P02.15 OPTIMIZING LENTIVIRAL VECTOR DESIGN AND PRODUCTION FOR CELL AND GENE THERAPIES

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10.1136/JITC-2026-ITOC.29

Lentivirus has become one of the most widely used viral vectors for gene delivery, owing to its high transduction efficiency, ability to infect both dividing and non-dividing cells, and capacity for stable, long-term transgene expression. Lentiviral vectors are used across a broad range of applications, from basic research to clinical settings, particularly for ex vivo modifications, with several FDA-approved therapies already in use. However, clinical translation remains challenging, with approximately 90% of cell and gene therapy trials failing, often due to suboptimal vector design that compromises efficacy, safety, and scalability. Although the gene of interest is crucial for therapeutic efficacy, optimization of the transfer plasmid backbone and helper plasmids is often overlooked, despite their significant impact on both efficacy and manufacturability. We demonstrate that rational vector design and optimized packaging protocols markedly enhance lentiviral vector pro-

duction, resulting in improved functionality, including robust CAR expression in T cells. Overall, our findings highlight the potential of optimizing lentiviral vector design and production as an effective strategy to advance cell and gene therapy development.

P02.16 CELL AVIDITY: THE NEXT-GEN BINDING ASSAY TO ADVANCE IMMUNE-BASED THERAPEUTIC DEVELOPMENT

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10.1136/JITC-2026-ITOC.30

Background While conventional assays such as affinity, cytokine secretion, and cytotoxicity provide valuable data at a molecular level, this information is insufficient to fully characterize and select the best cellular therapies. There is still a lack of understanding about the biophysical cell-cell interactions that drive functional processes.

Methods Cell avidity, the integrated strength of multivalent interactions between an effector cell and its target, can help elucidate the mechanism of action for therapeutic candidates. Our Cell Avidity platform challenges these interacting pairs using contactless force and quantifies the strength of binding between effector and target cells to distinguish productive from unproductive cell binding in a physiological context. This biophysical metric provides a unique view into cell binding characteristics to interrogate binding potency, selectivity, sensitivity, and kinetics.

Results Here, we review recent publications highlighting how researchers have used Cell Avidity to:

Fine-tune the affinity/cell avidity of CAR-T cells to mitigate on-target off-tumor toxicity in renal cell carcinoma.

Assess the contribution of CD58:CD2 in CAR mediated cell avidity and how replacing that with synthetic PD-1:CD2 interactions reduces fratricide while maintaining potency

Format-tune bispecific T cell engagers to enhance efficacy against renal cell carcinoma

Validate glyco-bridge binding in the context of CAR-T cells to overcome immunosuppressive tumor microenvironment

Engineer CAR-T cells secreting a T-cell engaging molecule to overcome a challenging tumor microenvironment in pancreatic adenocarcinoma.

Elucidate mechanism of action of tandem CAR-T targeting heterogenous solid tumors

Phenotype tumor-primed NK cells for cell binding and function

Conclusions We developed a Cell Avidity platform that enables the characterization and screening of molecular binders and cellular products, including antibodies, small molecules, and cell therapies. Cell Avidity provides essential information revealing potency, selectivity, sensitivity, and kinetics, offering key biophysical insights into the mechanism of action for immune-based therapies.

Poster Session 3. Immune Monitoring – Omics Technology

P03.01 IMMUNE MONITORING OF CLINICAL T-CELL RESPONSES IN ONCOLOGY IMMUNOTHERAPY USING TCR SEQUENCING AND TCR-EPITOPE ANNOTATION SOLUTIONS

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10.1136/JITC-2026-ITOC.31

Background T-cells form the basis of the current and next generation of cancer immunotherapeutics. There is however an unmet need to monitor if novel immunotherapies activate the immune system. Multi-omic sequencing approaches are being used to study T-cell populations and design more potent and effective therapies. T-cell receptor (TCR) sequencing in particular has emerged as an accurate and reproducible method for profiling T-cells, and is widely used in translational research in oncology. The TCR is crucial for T-cell functionality, defining the specificity of the immune response by recognizing peptide epitopes from cancer cells, presented by HLA molecules. TCR sequencing offers significant potential for immune monitoring and in-depth immune response analysis. Despite its advantages, such as ex vivo application, low sample volume requirements, and high sensitivity, interpreting TCR data remains a critical challenge.

We show that by using state-of-the-art TCR data analysis techniques, we can reliably identify immunotherapy-induced TCRs and as an immune monitoring strategy. We present use cases on Tumor-Infiltrating Lymphocyte (TIL) adoptive cell transfer (ACT) and on personalised neoantigen cancer vaccinations.

Methods We use TCR clustering and epitope-TCR annotation models such as ClusTCR and ImmuneWatch DETECT to identify immunotherapy-induced TCRs across different immuno-oncology applications. Based on state-of-the-art machine learning algorithms and a comprehensive database of known epitope-TCR interactions, these analyses rely on epitope-TCR pattern recognition and on the identification of TCR clonotypes that really matter.

Results Based on a published TIL study with TCR repertoire data from the T-cell product and from peripheral blood pre- and post-ACT, we show that the epitope-specificity of T-cell clusters in the TIL product can be determined in silico, both for tumor-associated and viral antigens. We show validation of the annotations in vitro using transgenic T-cells and epitope-specific stimulation experiments. In two independent neo-epitope cancer vaccination studies, we demonstrate the presence of vaccine-specific T-cells in TCR repertoires from blood and tumor

samples from vaccinated patients, and that these predictions correlate well with ELISPOT data. We show how directed generation of epitope-TCR training data can improve predictive performance for specific antigens of interest. Across the different applications, we also demonstrate that identified tumor-specific T-cells can be tracked in vivo using TCR sequencing as a powerful means of immune monitoring after immunotherapy.
Conclusion We provide an overview of several concrete TCR-based immune monitoring applications in the field of immunotherapy in oncology. TCR repertoire analysis is a novel and accessible methodology for monitoring T-cell responses against a broad range of antigens from a single blood sample. This methodology can be used to filter and identify clinically-relevant TCRs for use in therapy development, immune biomarker analysis, or the development of companion diagnostics.

MVH is an employee and SW, TB, PM are shareholders and directors of ImmuneWatch BV. All other authors: None.

P03.02 DECODING SINGLE-CELL PROTEIN INTERACTOMES OF CAR T CELLS WITH THE PROXIMITY NETWORK ASSAY

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10.1136/JITC-2026-ITOC.32

Proteins on the cell surface are key determinants of the cellular function in the adaptive immune system. The Proximity Network Assay (PNA) is a sequencing-based platform that resolves protein organization at single-cell resolution. Using DNA-barcoded antibodies and proximity-dependent ligation, PNA simultaneously measures the abundance, clustering, and colocalization of 155 immune proteins, generating nanoscale surface maps comprising ~50,000 molecular positions per cell without the use of optics. This spatially resolved readout enables systematic analysis of the membrane proteome across thousands of cells. We demonstrate the utility of PNA by identifying the proxiome of the CD19 CAR receptor at steady state and revealing dynamic proteomic remodeling during tumor cell encounter, including key phenomena such as trogocytosis and cell–cell conjugate formation that are key for CAR T cell function in vivo. By integrating spatial context with multiplex protein profiling at scale, PNA provides a powerful platform for protein interactomics, biomarker discovery, and mechanistic insights for cellular immunotherapies currently under clinical evaluation.

COI: I am a fully salaried employee of Pixelgen Technologies.

P03.03 DRIED ANTIBODY COCKTAILS: SETTING THE BENCHMARK FOR FAST AND ROBUST FLOW CYTOMETRIC ANALYSIS IN CELL AND GENE THERAPY

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10.1136/JITC-2026-ITOC.33

Flow cytometry is a key technology in the development, manufacturing, and quality assessment of cell and gene therapy (CGT) products. Notably within the CGT landscape is CAR T cell therapy, a major breakthrough in the treatment of hematological malignancies, for which flow cytometric analysis has become a versatile and indispensable tool. In clinical settings, flow cytometry is essential for patient monitoring, enabling the assessment of therapy effectiveness and facilitating timely adjustments to treatment plans. Ensuring comparability across treatment centers worldwide is vital for consistent patient care. However, variability in sample preparation and analytical execution continues to impede comparability. Numerous manual steps when using liquid antibodies contribute to variability, introducing operator-dependent errors and limiting reproducibility. Thus, meeting the growing demand for high-throughput, reliable, and reproducible data across laboratories and clinical environments still remains a major challenge.

We set out to demonstrate that dried antibody cocktails offer a promising solution. Our stable, ready-to-use reagents standardize staining workflows, reduce procedural complexity, enhance consistency, and improve efficiency of flow cytometric analysis for CGT applications. Cell material can be added directly to tubes containing the dried antibody cocktail, followed by a short incubation period prior to acquisition on a flow cytometer. This ready-to-use format reduces handling errors associated with conventional liquid antibody mixes.

Our experimental evaluation of dried antibody cocktails demonstrated a striking acceleration of the staining procedure for both experienced and inexperienced operators, along with low operator-dependent and day-to-day variability. Comparative experiments showed that staining results obtained with the dried format were highly comparable to those achieved using liquid antibodies, even for CAR T cell detection. Further assessments confirmed high lot-to-lot consistency and stable staining performance after extended storage at ambient conditions, highlighting the reproducibility and robustness of dried antibody cocktails. Considering the diverse range of applications, flexible panel selection has become increasingly important. We demonstrated that liquid antibodies or CAR detection reagents can easily be included in the staining procedure for a custom-tailored application. Moreover, custom manufacturing of dried antibody cocktails allows for fully flexible panel design to meet specific experimental or clinical requirements. Additionally, the ability to customize quality control testing ensures reliability and consistency.

In conclusion, dried antibody cocktails streamline flow cytometry workflows, making analysis faster and more robust for both clinical and research applications. They improve global comparability and efficiency in CGT manufacturing and enable uniform patient monitoring across treatment centers.

All authors: significant

Poster Session 4. Immunooncology in gynecological cancers

P04.01 REDUCED HLA DIVERSITY AND IMPAIRED IMMUNE SURVEILLANCE IN VERY EARLY-ONSET OVARIAN CANCER PATIENTS

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10.1136/JITC-2026-ITOC.34

Background Reduced diversity at human leukocyte antigen (HLA) loci may compromise immune recognition of tumor neoantigens. We hypothesized that decreased heterozygosity at HLA loci is associated with an increased risk of developing very early onset ovarian cancer (OC). Unlike late-onset OC, OC diagnosed before 30 years of age is rarely attributable to germline pathogenic variants (GPV) in established high-to-moderate-penetrance OC predisposition genes, suggesting alternative mechanisms of genetic susceptibility, including immune-related factors.

Methods We performed a comprehensive germline analysis of 123 early-onset OC patients using DNA and RNA whole-exome sequencing, complemented by HLA genotyping and polygenic risk score analyses. Genetic findings were evaluated in clinical and histopathological context and compared with multiple control cohorts, including 378 population-matched controls, 227 non-cancer female controls, and 5099 unselected individuals from Czech National Marrow Donors Registry.

Results HLA analysis revealed a significantly increased frequency of HLA homozygosity in early-onset OC patients compared with controls, affecting both class I and class II loci. We also observed a significant enrichment of specific class I (HLA-A*36:01, HLA-B*53:01) and II (HLA-DRB1*11:01, HLA-DQA1*01:03) alleles. Gene burden analysis further identified enrichment of GPV in immune-related genes, notably LY75-CD302, which encode receptors involved in antigen uptake and HLA class I–mediated antigen presentation.

Conclusions Early-onset OC is characterized by a distinct and highly heterogeneous germline genetic architecture. Our findings implicate impaired immune surveillance—reflected by reduced HLA diversity and enrichment of immune-related GPV—as a potential contributor to disease susceptibility.

Supported by the Ministry of Health of the Czech Republic: DRO-VFN-00064165; Charles University: COOPERATIO, UNCE/24/MED/022; and the Ministry of Education Youth and Sports of the Czech Republic: MULTIOMICS_CZ (Programme Johannes Amos Comenius, ID Project CZ.02.01.01/00/23_020/0008540) – Co-funded by the European Union.

P04.02 MEMBRANE-BOUND CX3CL1 ENHANCES NK CELL–MEDIATED CYTOTOXICITY IN OVARIAN CANCER

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10.1136/JITC-2026-ITOC.35

Background The chemokine CX3CL1 (fractalkine) is a unique member of the chemokine family that exists as both a soluble and a membrane-bound molecule and exhibits distinct functions in cancer. Proteolytic shedding of membrane-bound CX3CL1 (mCX3CL1) by the metalloproteases ADAM10 and ADAM17 generates soluble CX3CL1 (sCX3CL1), which primarily mediates chemotactic immune cell recruitment, whereas the membrane-bound form is implicated in cell–cell adhesion, migration, and direct activation of cytotoxic lymphocytes. CX3CL1 has been described to display both tumor-suppressive and tumor-promoting properties depending on tumor entity and microenvironment and is a known modulator of natural killer (NK) cell recruitment, activation, and effector function via its receptor CX3CR1. Despite increasing evidence for a context-dependent role of the CX3CL1/CX3CR1 axis in cancer, the individual contributions of soluble versus membrane-bound CX3CL1 have not been systematically addressed in ovarian cancer. In this study, we specifically examine the aspect of NK cell cytotoxicity and whether increasing tumor cell–associated mCX3CL1, either through inhibition of ADAM10/17-mediated shedding or enforced CX3CL1 expression, can enhance NK cell–mediated cytotoxicity.

Methods Human serous ovarian carcinoma cell lines OV-MZ-6 and OVCAR-3 were stimulated with the inflammatory cytokine TNF-α and/or treated with selective ADAM10 (GI-254023X) or ADAM17 (TAPI-2) inhibitors. CX3CL1 expression was quantified by ELISA for soluble CX3CL1 and by flow cytometry for membrane-bound CX3CL1. Transient overexpression of CX3CL1 was achieved using a pCMV6-CX3CL1 expression vector. NK cell–mediated cytotoxicity was assessed using a europium-based DELFIA cytotoxicity assay at multiple effector-to-target ratios. CX3CL1 dependency was further evaluated by antibody-mediated blockade during cytotoxicity assays.

Results In OV-MZ-6 cells, TNF-α stimulation markedly increased CX3CL1 expression and was associated with enhanced NK cell–mediated cytotoxicity. Antibody-mediated blockade of CX3CL1 partially abrogated the enhanced cytotoxicity, indicating a functional contribution of tumor cell–associated mCX3CL1. Inhibition of ADAM17 significantly reduced the release of soluble CX3CL1 while concomitantly increasing membrane-bound CX3CL1, as demonstrated by flow cytometry. This resulted in a significant enhancement of NK cell–mediated lysis. In contrast, ADAM10 inhibition led to a lesser reduction of soluble CX3CL1 and a less pronounced increase in mCX3CL1 and showed no significant effect on NK cell–mediated lysis. Transient overexpression of CX3CL1 significantly increased NK cell–mediated lysis in OV-MZ-6 cells, whereas

additional ADAM17 inhibition did not further augment cytotoxicity. In OVCAR-3 cells, TNF-α induced only minimal CX3CL1 expression and did not enhance NK cell–mediated lysis; however, CX3CL1 overexpression resulted in a non-significant trend toward increased cytotoxicity.

Conclusion Membrane-bound CX3CL1 enhances NK cell–mediated cytotoxicity against ovarian cancer cells in a cell line–dependent manner. ADAM17 inhibition may represent a strategy to increase mCX3CL1 and improve NK cell–mediated tumor cell lysis in vitro. Further studies are required to understand the distinct functional roles of membrane-bound and soluble CX3CL1 and to explore their potential for future pharmacological exploitation.

Poster Session 5. Mode of Action of Immune Therapies

P05.01 DYNAMIC MODULATION OF NATURAL KILLER (NK) AND CYTOKINE INDUCED KILLER (CIK) CELLS BY EXTRACORPOREAL PHOTOPHERESIS IN CHRONIC GRAFT-VERSUSHOST DISEASE AFTER HEMATOPOETIC STEM CELL TRANSPLANTATION

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10.1136/JITC-2026-ITOC.36

Background NK cells are central to immune reconstitution after allo-SCT and contribute to GVHD pathophysiology. Altered NK maturation and inhibitory phenotypes have been linked to persistent inflammation in cGVHD. ECP is widely used in steroid-refractory cGVHD, but its effects on NK-cell subset composition remain poorly defined.

Aims To compare NK and checkpoint-expressing NK/CIK subsets between patients without GvHD ('No-GvHD', controls) and cGVHD patients receiving ECP at baseline and after 12 weeks.

Methods ECP was administered using the THERAKOSTM CELLEXTM Photophoresis system. Peripheral Blood samples from cGVHD (n=14) and no-GVHD (n=14) allo-SCT recipients were analyzed at baseline and after 12-weeks follow-up. NK and CIK subsets were quantified by flow cytometry. Statistical analyses were performed.

Results

1. cGVHD displayed vs no-GvHD (baseline):
   ↑Cytokine-producing NK (CD56bri) cells
   ↓TIGIT-expressing NK cells
   ↓KLRG1-expressing and TIM3-expressing CIK cells
2. After 12 weeks from baseline, cGVHD patients displayed vs no-GvHD:
   ↓Cytotoxic NK ($CD56dimCD16^+$, $CD56dimCD57^+$) cells
   ↓TIGIT-expressing and KLRG1-expressing NK cells
   ↓KLRG1-expressing CIK cells
3. cGVHD patients displayed after 12 weeks ECP treatment:
   ↓Cytokine-producing NK (CD56bri) cells
   ↑TIGIT-expressing, KLRG1-expressing NK cells and $2B4^+$ expressing NK cells
   ↓TIGIT -expressing and KLRG1-expressing CIK cells

Conclusions cGVHD patients showed reduced cytotoxic NK signatures at follow-up compared with no-GVHD controls. During ECP, NK cells shifted toward increased inhibitory/stimulatory phenotypes and reduced cytokine-producing subsets, while CIK cells displayed an opposite trend. These data suggest differential NK/CIK remodeling as part of ECP's immunomodulatory mechanism. Clinical correlations will be presented at the meeting.

Funding This work was funded by an external collaborative research (ECR) grant provided by Therakos EMEA Limited

P05.02 CHARACTERIZING BISPECIFIC T-CELL ENGAGERS WITH ENGINEERED CD3 EFFECTOR AND TAILORED TARGET CELLS

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10.1136/JITC-2026-ITOC.37

Backround Bi- and multi-specific modalities, with their numerous formats, are advancing cancer immunotherapy, particularly in hematologic malignancies. Created from components like immunoglobulin half molecules, Fab fragments, or single chain Fvs, they can recruit and activate immune cells or interfere with receptor signalling. Hundreds of such antibodies are in clinical development, demonstrating various modes of action. Blinatumomab, a Bispecific T-cell Engager (BiTE) molecule, targets CD3 and CD19 for T-cell mediated elimination of B-cell malignancies. CD19 is an attractive target because early B-cell malignancies arise from CD20-negative Pro-B and Pre-B cells, unlike Rituximab, which targets CD20. Consequently, blinatumomab was approved for aggressive B-cell precursor acute lymphoblastic leukemias (ALLs), often affecting children. This success has spurred development of other bispecific molecules for hematologic malignancies, with over two-thirds targeting CD19 and CD20 and a majority targeting CD3 on the effector side.

Methods We engineered and cloned a reporter cell line based on the Jurkat human T-cell line, by stably expressing CD3 and a CD3-signaling responsive, NFAT-driven Firefly Luciferase reporter gene. Upon selection of the best performing clone a Renilla Luciferase expression construct under the control of a constitutively active promoter was added, allowing to assess potential toxic effect and normalization of the assay results. Based on the final clone, cell production was optimized to provide the basis for an unlimited source of frozen, ready-to-use cells.

Results Using the BiTE Blinatumomab, we showcase that our above-described CD3-responsive effector cells reply in a specific and dose responsive manner to the presence of Blinatumomab and CD19 positive target cells. In addition, we demonstrate the assay performance and robustness in different application settings.

Conclusion Analytical methods for BiTEs based on primary cells have limitations which can be overcome with our iLite® bioassay platform allowing to assess CD3-dependent MoAs in an easy and reliable way. Despite not shown here, the cells were proven to work also in the context of a variety of BiTEs directed against a multitude of different antigen targets. Beyond that, this cell line can be a starting point for further cell line engi-

neering to reflect additional MoAs on top of CD3-based T-cell engagement of more complex modalities. The user-friendly, frozen, Assay Ready Cell format allows a maximum of flexibility while providing highest assay consistency in the contexts of drug screening and characterization as well as in drug potency and immunogenicity assessment.

All authors are employees of Svar Life Science

Poster Session 6. New Targets and New Leads

P06.01 SPATIAL AND SINGLE-CELL PROFILING REVEALS STROMAL REGULATION OF NK-CELL CYTOTOXICITY IN GALLBLADDER CANCER

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10.1136/JITC-2026-ITOC.38

Background Gallbladder cancer (GBC) is a highly lethal malignancy with limited responsiveness to immunotherapy. Natural killer (NK) cells are essential innate immune effectors responsible for tumor surveillance; however, how their cytotoxic activity is regulated within the unique tumor microenvironment (TME) of GBC remains largely unknown. Understanding NK-cell dysfunction in GBC may provide new insights into mechanisms of immune escape and therapeutic resistance.
Methods We integrated single-cell RNA sequencing and spatial transcriptomic profiling to characterize NK-cell heterogeneity and its cellular and molecular regulation in GBC. CellChat-based cell–cell communication analysis, spatial co-localization assessment, and multiplex immunofluorescence were conducted to identify regulatory interactions and validate spatial associations. Spatial transcriptomics further enabled the identification of an immunosuppressive ecological niche in which NK-cell cytotoxicity was markedly reduced compared with immune-active niches.
Results A tumor-associated NK (TaNK) subset exhibiting impaired cytotoxicity was identified in GBC. Myofibroblastic cancer-associated fibroblasts (myCAFs) emerged as dominant regulators of NK-cell dysfunction, interacting strongly with TaNK cells through the COL1A1–LAIR1 ligand–receptor axis. This signaling pathway represents a key mechanistic link connecting stromal remodeling to NK-cell suppression. Spatial transcriptomic mapping and histological validation confirmed the co-localization of myCAFs and TaNK cells, particularly within an immunosuppressive niche enriched in extracellular matrix components and immune-exclusion features. Tumors with high co-infiltration of myCAFs and TaNKs displayed pronounced immune exclusion and were associated with unfavorable immunotherapy outcomes. NK cells residing in the immunosuppressive niche demonstrated markedly reduced cytotoxic gene expression and elevated exhaustion signatures, highlighting niche-specific functional impairment.
Conclusions Our study reveals that myCAF-derived COL1A1–LAIR1 signaling plays a crucial role in suppressing NK-cell cytotoxicity and fostering the development of an immunosuppressive ecological niche in GBC. By integrating single-cell and spatial analyses, we delineate a stromal–immune interaction axis that contributes to immune evasion and immunotherapy resistance. These findings provide mechanistic insights into NK-cell dysfunction in GBC and identify potential therapeutic targets for improving immunotherapy efficacy in this highly aggressive cancer.

The authors have no relevant financial or non-financial conflicts of interest to disclose.

P06.02 ABSTRACT WITHDRAWN

P06.03 NEXT-GENERATION TARGETED IMMUNOMODULATORY PROTEIN FOR HUMAN PROSTATE CANCER

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10.1136/JITC-2026-ITOC.39

Background Immunotherapy explores multiple strategies to enhance the host immune system's ability to detect and eliminate cancer cells.[1] Most immunotherapeutic approaches aim to counteract tumor-induced immunosuppressive mechanisms or to potentiate antitumor immunity through co-stimulation of T-cell receptors.[2] Members of the tumor necrosis factor superfamily (TNFSF) play a central role in promoting T-cell proliferation and differentiation, thereby enhancing immune effector functions. The 4-1BB receptor is a member of this family, and its costimulation using agonistic antibodies has demonstrated potent antitumor effects; however, this strategy has been associated with hepatic toxicity, likely due to systemic receptor activation.[3] Here, we propose a novel targeted platform for 4-1BB receptor costimulation, engineering chimeric recombinant proteins composed of a tumor receptor–binding domain (LD) fused to a functional 4-1BBL ligand domain (4-1BBL).
Methods Chimeric proteins were produced using baculovirus/insect cell expression system and purified by immobilized metal affinity chromatography (IMAC) followed by ion-exchange chromatography. Protein characterization was carried out by Western blot and flow cytometry. The ability of the chimeric proteins to selectively binding PSMA-positive cells was examined by fluorescence microscopy using a LD/GFP fusion protein. In vitro functional assays were performed using Peripheral Blood Mononuclear Cells (PBMCs) obtained from healthy donors. PBMC proliferation was evaluated by CFSE dilution, and tumor cell cytotoxicity was assessed in co-culture assays with PSMA-expressing PC-3 cells. PBMCs were pre-activated with anti-CD3 antibodies and subsequently treated with the recombinant protein LD/4-1BBL, to evaluate T cell costimulation. For in vivo studies, NOD-SCID mice were subcutaneously engrafted with PBMCs and PSMA expressing PC-3 cells, following intraperitoneal administration of LD/4-1BBL on days 1, 3, and 7 post-inoculation.
Results Fluorescence microscopy revealed increased accumulation of LD/GFP proteins in PSMA-expressing cells compared to receptor-negative cells, confirming the targeting specificity driven by LD ligand. In vitro assays showed that the LD/4-1BBL significantly enhanced PBMC proliferation. Additionally, treatment with LD/4-1BBL promoted increased tumor cell cytotoxicity and elevated IFN-γ secretion. In vivo administration of LD/4-1BBL resulted in a marked reduction in PC-3 PSMA tumor growth.

Conclusion These results demonstrate that the chimeric LD/4-1BBL protein represents a promising targeted immunotherapeutic strategy by selectively directing 4-1BB costimulation to PSMA-positive tumor cells, leading to robust antitumor activity in both in vitro and in vivo models.

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P06.04 INTEGRATED DE NOVO DESIGN AND HIGH-THROUGHPUT SCREENING OF T CELL RECEPTOR MIMETIC MINIBINDERS FOR MULTIPRONGED INTRACELLULAR CANCER ANTIGEN TARGETING

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10.1136/JITC-2026-ITOC.40

Background Intracellular antigens have remained beyond the reach of most immunotherapies due to limited accessibility. To overcome this limitation, we applied artificial intelligence (AI)–guided protein design to generate peptide–major histocompatibility complex (pMHC) class I–specific minibinders that mimic T cell receptor (TCR) recognition. Previous AI-designed pMHC binders, including our own, have primarily focused on high specificity. However, natural TCRs are inherently cross-reactive, enabling recognition of multiple tumor-associated antigens (TAAs) through a single TCR. Notably, multipronged T cells were shown to enhance tumor recognition across cancer types.[1] We therefore investigated whether AI-driven design can intentionally engineer multipronged pMHC minibinders. As a proof of principle, we targeted three TAA–derived (Melan A, BST2, IMP2) peptides that are known to be cross-reactively recognized in the context of HLA-A02:01.[1]

Material and Methods Candidate minibinders were generated using RFdiffusion[2], BindCraft[3], and BoltzGen[4], followed by iterative refinement of RFdiffusion and BindCraft designs using a custom refinement algorithm, yielding a total of 12,000 designs. All candidates were cloned as an oligonucleotide pool and screened in parallel using the transposon display platform.[5] Enriched binders were identified by next-generation sequencing.

Results Screening yielded target-specific success rates of 0.4–6.1%. Among 375 enriched minibinders, 20 demonstrated multipronged binding to at least two pMHC targets. Complementary in silico analyses were performed to assess which metrics best predict multipronged design success. Ongoing validation aims at testing soluble binders and chimeric antigen receptor T cell and bi-specific T cell engager formats, while our comprehensive in silico database supports future metric refinement.

Conclusion This work introduces a paradigm shift in binder design, in which multipronged cross-reactivity is intentionally engineered as a therapeutic advantage. By targeting multiple TAAs, such minibinders may mitigate antigen escape, reduce resistance, and enable pan-cancer applicability through conserved peptide motifs, while enhancing sensitivity in low-antigen-density tumors. We establish a proof of concept for the intentional design of multipronged pMHC binders and outline both the potential and current limitations of de novo TCR-mimetic design approaches.

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P06.05 DE NOVO DESIGNED PROTEIN BINDERS AGAINST CANCER TARGETS OF THE B7-H FAMILY

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10.1136/JITC-2026-ITOC.41

Background Artificial intelligence-guided protein design offers a powerful approach for developing novel therapeutic agents, particularly in cases where conventional antibody or single-domain antibody development proves challenging. However, computational predictions have to be carefully considered and need to be validated for target affinity and specificity. Suitable targets for such a platform establishment are well known molecules, such as PD-L1, and closely related proteins, here comprising CD276 and VTCN1 from the same B7-H family.

Material and Methods Using RFdiffusion, a machine learning-based pipeline derived from RoseTTAFold, we generated libraries of de novo designed proteins targeting members of the B7-H family to develop a screening and validation pipeline. Using a mammalian cell surface system, we screened thousands of potential protein binders for their target recognition. Further validation and characterization of binder subsets was performed using flow cytometry, chimeric antigen receptor (CAR)-T cells, and surface plasmon resonance (SPR) spectroscopy.

Results We established a high-throughput, cell-surface expression-based screening platform capable of selecting target-specific binders from libraries comprising thousands of candidates. In a first approach, several binders against PD-L1 (B7-H1) and CD276 (B7-H3) were identified and validated via flow cytometry, SPR spectroscopy, and interface mutant analysis. These mini-proteins demonstrated high target affinity, small molecular size, and remarkable thermal stability, making them versatile tools for biomedical and clinical applications. Using CAR-T cells, we were able to test the binders' functionality and target specificity in a clinically relevant setting.

Conclusion Altogether, this platform offers a versatile and scalable framework for the rapid development of de novo designed proteins with potential applications in research, diagnostics, and therapy.

P06.06 CLEVER-1 BLOCKADE AS A POTENTIAL IMMUNOMODULATORY TREATMENT FOR BRAIN TUMORS

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10.1136/JITC-2026-ITOC.42

Background Common lymphatic endothelial and vascular endothelial receptor-1 (Clever-1) is a scavenger receptor expressed primarily by lymphatic and sinusoidal endothelial cells and immunosuppressive macrophages.[1] Clever-1 expression is upregulated in several types of cancer and is associated with poor prognosis. Bexmarilimab, an inhibitory antibody targeting Clever-1, has demonstrated clinical benefit in subsets of patients with advanced solid malignancies by reprogramming immunosuppressive macrophages, enhancing their antigen presentation, and activating adaptive immunity.[2] Initial survival analyses of patient-derived brain tumor tissues stained for Clever-1 expression revealed a strong correlation between higher Clever-1 expression and poor overall and progression-free survival. Based on these findings, our study investigates the potential of macrophage reprogramming with bexmarilimab as an immunomodulatory treatment for pediatric and adult brain tumors, that are characterized by a macrophage-rich tumor microenvironment (TME).

Material and Methods We used patient derived explant cultures (PDEC) derived from primary brain tumor tissues including glioblastoma (n=3), medulloblastoma (n=1), astrocytoma (n=7), oligodendroglioma (n=2), meningioma (n=2), atypical teratoid/rhabdoid tumor (n=1), and brain squamous cell carcinoma (n=1) and treated them with Bexmarilimab (50μg/mL) and its isotype control IgG4 (50μg/mL) for 16 hours. Following the treatment, we performed bulk RNA-sequencing, immunofluorescence staining and multiplex cytokine analyses with the PDECs to assess the impact of Clever-1 inhibition on the brain TME.

Results Immunofluorescence staining of the PDECs showed that Clever-1 is predominantly expressed on the myeloid cell populations within the brain TME. Using a core response gene signature derived from clinical data of patients who responded favourably to Bexmarilimab treatment,[3] we distinctly identified potential responders and non-responders amongst the treated PDECs of brain tumors. Preliminary findings suggested transcriptomic and cytokine profile changes in response to Bexmarilimab treatment compared to the isotype control treatment, with key immune pathways activated in responsive samples. These responders exhibited low IFN signalling at baseline while Bexmarilimab induced pro-inflammatory immune responses implying enhanced antigen-presentation, upregulated IFN-γ, IFN-α and TNF-α signalling and downregulated M2 macrophage signature.

Conclusion Our findings support Bexmarilimab as a promising treatment to induce antitumor responses by reprogramming immunosuppressive macrophages in pediatric and adult brain tumors and provide a means to predict the efficacy of Bexmarilimab using patient-derived tumor tissues.

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M. Hollmén: Faron Pharmaceuticals Ltd, Turku, Finland: significant, R. Suresh: None, R. Turpin: None, K. Nordfors: None, M. Mieskolainen: None, I. Hermelo: None, J. Kolivuori: None, H. Haapasalo: None, J. Haapasalo: None

P06.07 TARGETING IMMUNE CHECKPOINT PROTEINS CD47 AND GALECTIN-3 AND -9 TO IMPROVE MACROPHAGE MEDIATED PHAGOCYTOSIS OF PANCREATIC TUMOR CELLS

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10.1136/JITC-2026-ITOC.43

Background Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumor entities, with steadily increasing incidence. The tumor microenvironment (TME) is characterized by an intensely immunosuppressive stroma, which comprises up to 90% of the tumor volume. Tumor-associated macrophages (TAMs) are the dominant immune cell population in PDAC and mostly exhibit a M2-like phenotype supporting the immunosuppressive milieu as well as tumor progression. Similar to several entities, the immune checkpoint molecule CD47 is upregulated in PDAC and correlates with advanced disease stages and poor outcomes. CD47 mediates a 'don't eat me' signal to macrophages after binding to signal regulatory protein α (SIRPα), serving as an immune escape mechanism by evading phagocytosis. Additionally, lectins like galectin-3 and -9 are emerging as potential immune checkpoint targets as they are promoting tumor progression and immunosuppression by impacting immune cell differentiation and activity. Therefore, this study aimed at targeting CD47 as well as galectin-3 and -9 to improve PDAC cell phagocytosis by macrophages.

Methods Monocytes were isolated from peripheral blood of healthy donors and differentiated into M1- and M2-like macrophages. Multiple PDAC cell lines as well as M1- and M2-like macrophages were characterized regarding their immune checkpoint expression and localisation. PDAC cell phagocytosis by M1- or M2-like macrophages was investigated over time using a pH dependent live cell stain and automated microscopy. Furthermore, the impact of CD47, galectin-3 or galectin-9 antibody-mediated blockade on phagocytosis of PDAC cells was evaluated.

Results The immune checkpoint molecules CD47 as well as galectin-3 and -9 were heterogeneously expressed by PDAC cells. Overall, expression of galectins was higher in macrophages compared to tumor cells and differed tremendously between M1- and M2-like macrophages. Phagocytosis of tumor cells by

M2-like macrophages varied depending on the PDAC cell line, while M1-like macrophages exhibited low to no phagocytotic potential. Furthermore, antibody-mediated blockade of CD47 significantly increased phagocytosis of tumor cells by M2-like but not M1-like macrophages, while blocking of galectins did not significantly impact phagocytosis rates.
Conclusion Although CD47 blockade significantly increased phagocytosis, many PDAC cells remained untargeted by M2-like macrophages and blocking of galectins did not impact M2-like macrophage mediated phagocytosis of PDAC cells, indicating that blocking of CD47 or galectins alone is not sufficient to induce elimination of PDAC cells. These findings suggest that regulation of phagocytosis is not depended on CD47 exclusively, thus multiple factors may need to be targeted simultaneously. As galectin-3 has been described to cooperate with CD47 in suppressing phagocytosis in other entities, combination of both strategies should be further investigated.

P06.08 TARGETING UXS1 TO SUPPRESS COLORECTAL CANCER PROGRESSION BY REGULATING GLYCAN BIOSYNTHESIS, METABOLIC REPROGRAMMING, AND IMMUNE EVASION

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10.1136/JITC-2026-ITOC.44

Background Colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide, with recurrence and metastasis posing major therapeutic challenges. UDP-xylose synthase 1 (UXS1) generates UDP-xylose required for initiation of glycosaminoglycan (GAG) biosynthesis, yet its role in CRC progression is poorly understood.
Material and Methods Public transcriptomic datasets were analyzed to assess UXS1 expression and clinical relevance. Genetic ablation of UXS1 was performed using knockout or knockdown approaches in colon cancer cell lines. Metabolic profiling, cell surface GAG analysis, RNA sequencing, and ferroptosis assays were conducted. Tumor growth and immune infiltration were evaluated in syngeneic mouse models. Structure-based molecular docking was used to identify candidate UXS1 inhibitors.
Results UXS1 expression was elevated in CRC tissues and associated with poor patient prognosis. UXS1 knockout or knockdown impaired colon cancer cell viability and invasiveness, independent of UDP-glucose dehydrogenase expression. Mechanistically, loss of UXS1 reduced cell surface GAG levels, suppressed glycolytic and tricarboxylic acid cycle activity, and increased ferroptosis, consistent with RNA sequencing analyses. In syngeneic models, Uxs1 knockout markedly inhibited tumor growth in CT26 tumors. Notably, Uxs1-deficient CT26 tumors displayed increased leukocyte infiltration, indicating a previously unrecognized immunomodulatory function. Molecular docking identified pemetrexed as a putative UXS1 inhibitor, and pemetrexed treatment reduced cell surface GAG expression, supporting direct pharmacologic targeting of UXS1.
Conclusions These findings suggest that altered glycan biosynthesis, metabolic reprogramming, and immune suppression collectively contribute to UXS1-mediated colorectal cancer progression and establish UXS1 as a promising therapeutic target.

P06.09 SEQUENTIAL PHOTODYNAMIC THERAPY AND ALPHAVIRUS-MEDIATED INTERFERON-Γ DELIVERY REMODEL THE TUMOUR MICROENVIRONMENT IN BREAST CANCER

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10.1136/JITC-2026-ITOC.45

Background Breast tumours with an immunosuppressive microenvironment respond poorly to immunotherapy, highlighting the need for strategies that simultaneously induce tumour cell death and reprogram tumour-associated immune cells. Photodynamic therapy (PDT) induces local oxidative stress and immunogenic cell death, while virus-based cytokine gene delivery offers a means to modulate immune function directly within the tumour microenvironment. However, the therapeutic interplay between PDT and virus-mediated cytokine delivery remains largely unexplored. This study investigates the combined potential of chlorin e6–based PDT and replication-deficient Semliki Forest virus–mediated interferon-γ (IFNγ) delivery for immune remodelling in breast cancer.
Methods Murine 4T1 breast cancer cells were analysed in 2D cultures and 3D spheroid models to assess treatment-induced cytotoxicity, immunogenic cell death markers, viral transgene expression, and macrophage phagocytosis. Treatment sequencing was systematically evaluated to define optimal conditions for combination therapy. Therapeutic efficacy and immune modulation were further assessed in an orthotopic, immunocompetent 4T1 mouse model using sequential PDT and intratumoral viral administration. Tumour burden was quantified, and immune cell composition was analysed using high-dimensional spectral flow cytometry.
Results PDT induced reactive oxygen species production and immunogenic tumour cell death, enhancing macrophage-mediated phagocytosis but not directly driving macrophage polarisation. In 3D tumour spheroids, treatment order was critical: PDT impaired viral gene expression when applied first, whereas viral priming followed by PDT resulted in pronounced synergistic tumour cell killing. In vivo, sequential PDT followed by IFNγ gene delivery significantly reduced tumour burden compared with either monotherapy. Immune profiling revealed that PDT primarily activated innate immunity, increasing dendritic cells, natural killer cells, and iNOS-positive myeloid populations, while IFNγ delivery reduced immunosuppressive cell subsets, including regulatory T cells, CD206-positive macrophages, and myeloid-derived suppressor cells.
Conclusions This study demonstrates that rationally sequenced PDT and virus-mediated IFNγ delivery synergistically remodel the breast tumour microenvironment toward an immunostimulatory state. The findings underscore the importance of treatment timing and highlight innate immune activation as a key mechanism driving therapeutic efficacy, supporting further development of combined photo-immunogene strategies for resistant breast cancers.

P06.10 TARGETING GALE-MEDIATED NUCLEOTIDE SUGAR HOMEOSTASIS SUPPRESSES BREAST CANCER CELL INVASIVE BEHAVIOR

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10.1136/JITC-2026-ITOC.46

Background Breast cancer progression requires coordinated regulation of glycosylation and cellular metabolism, yet the molecular mechanisms integrating these processes to drive aggressive disease remain poorly defined. UDP-galactose 4-epimerase (GALE), which interconverts UDP-galactose/UDP-GalNAc and UDP-glucose/UDP-GlcNAc, represents a central node controlling nucleotide-sugar homeostasis that is essential for both glycosylation and cellular metabolism.
Material and Methods Public breast cancer transcriptomic datasets were analyzed to assess GALE expression, patient survival, and immune cell infiltration across molecular subtypes. GALE function was examined using genetic depletion in breast cancer cell lines followed by migration and invasion assays, together with Seahorse analyses of glycolytic activity and mitochondrial respiration. GALE-dependent glycosylation remodeling was characterized by examining Golgi-mediated glycosylation and intracellular protein O-GlcNAcylation. Molecular docking was performed to identify candidate GALE-binding compounds.
Results GALE was significantly upregulated in breast tumors, and high GALE expression was associated with poor overall survival in both triple-negative and $ER^+/PR^+/HER2^-$ subtypes. Genetic depletion of GALE markedly suppressed breast cancer cell migration and invasion and impaired both glycolytic activity and mitochondrial respiration, indicating an essential role in maintaining metabolic fitness. Mechanistically, GALE depletion reduced UDP-GlcNAc availability, leading to global suppression of protein O-GlcNAcylation, while concomitantly increasing UDP-galactose levels and inducing Golgi-mediated glycosylation remodeling characterized by enhanced terminal β-galactosylation and LacNAc extension. Immune profiling revealed subtype-specific associations: in triple-negative breast cancer, high GALE expression was associated with reduced cytotoxic $CD8^+$ T-cell infiltration, consistent with an immune-excluded phenotype; in contrast, in $ER^+/PR^+/HER2^-$ breast cancer, GALE expression correlated with Th1-related immune signaling without increased $CD8^+$ T-cell infiltration, indicative of an immune-quiescent microenvironment. Structure-based molecular docking identified multiple clinically relevant compounds with predicted GALE-binding activity.
Conclusions GALE acts as a central nucleotide-sugar homeostasis regulator that coordinates glycan remodeling, metabolic adaptation, and immune contexture in breast cancer. These findings establish GALE as a druggable glyco-metabolic node whose inhibition may suppress breast cancer invasiveness and modulate immune exclusion, supporting GALE as a promising therapeutic target.

P06.11 EXPLORATION OF THE INVOLVEMENT OF RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS (RAGE) SIGNALING IN THE REGULATION OF TUMOR IMMUNITY BY ISOLIQUIRITIGENIN IN PANCREATIC CANCER CELLS

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10.1136/JITC-2026-ITOC.47

Background We have recently confirmed that the novel autophagy regulator isoliquiritigenin (ISL) could act as inhibitor of the KRAS downstream executor Rac1 in treating pancreatic cancer. Receptor of Advanced Glycation End production (RAGE), another upstream regulator of Rac1, is a member of the immunoglobulin superfamily that is overexpressed during cancer development in the tumor microenvironment (TME). In this study, we have investigated the involvement of RAGE in the anti-neoplastic activity of ISL in association with regulation of the tumor immunity in pancreatic ductal adenocarcinoma (PDAC) cells.
Materials and Methods Cell migratory activity was determined in PANC1 PDAC cells by using Wound healing assay. Protein expression of various tumor immunity biomarkers and metastatic mediators were determined by Western immunoblotting.
Results We recently reported that ISL inhibited the protein expression of Rac1 and EMT/pro-migratory biomarkers in PDAC cells. Molecular docking and dynamic simulation tests had confirmed that ISL has a strong covalent binding and active interaction with RAGE. In both PANC and MIA-PaCa2 PDAC cells, ISL downregulated both gene and protein expression of RAGE and its ligand HMGB1, with concurrent upregulation of PTEN and mTOR (in PI3K-Akt pathway). ISL also decreased the protein expression of JAK1, STAT3, STAT1, p-STAT1 as well as the anti-apoptotic factor BCL-xL in PANC-1 cells. RAGE and JAK-STAT signaling work together to affect T-cell infiltration and is involved in tumor immunosuppression in the TME. Here, we discovered that ISL reduced the protein expression of CD63 (critical for mediating KRAS-mutated PDAC cell response to macrophage induction) and CD68 (known to regulate tumor immunity including CD4+ and CD8+ T cells in the TME as immune checkpoint in cancer treatments to enhance cell proliferation and migration). Alternatively, CD26 protein expression was increased only modestly by ISL. Since serum CD26 level was proposed to be associated with favorable clinicopathological features in pancreatic cancer patients, its gene expression level in tumor and serum of xenografted animals following ISL treatment needs to be measured to verify its implication. Our preliminary studies have indicated that CD9 (expressed in tumor-initiating cells or cancer stem cells of pancreatic cancer) and CD98 (transmembrane protein acting as driver of integrin-dependent metastasis) levels were found to be reduced slightly by ISL. We will detect their gene expression levels to confirm our findings.

Conclusion Our results have revealed that ISL suppressed EMT and migratory activity in PDAC cells by inhibition of RAGE involving restoration of apoptosis through modulation of JAK-STAT and PI3K-Akt-mTOR signaling. ISL may also act by regulation of tumor immunity, directly or via RAGE inhibition, as inhibitor that neutralizes CD63 and CD68 in the TME of PDAC, providing a new direction for tumor immunotherapy.

P06.12 PCIF1 CORRELATES WITH IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT AND PREDICTS RESPONSE TO IMMUNE CHECKPOINT BLOCKADE THERAPY IN HEPATOCELLULAR CARCINOMA

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10.1136/JITC-2026-ITOC.48

Background Hepatocellular carcinoma (HCC) remains a global health burden with high morbidity and mortality. The response rate to immunotherapy in HCC patients remains limited to 15-30%, underscoring the clinical urgency for reliable predictive biomarkers to guide treatment decisions. While Phosphorylated CTD Interaction Factor 1 (PCIF1), a unique m6Am methyltransferase, has been implicated in tumorigenesis, its functional role in regulating immune landscape and predicting immune checkpoint blockade (ICB) efficacy in HCC remains unclear.

Methods TCGA database analysis was performed to explore PCIF1 expression and its correlation with immune cell infiltration and Tumor Immune Dysfunction and Exclusion (TIDE) scores. A total of 245 patients with advanced HCCs were enrolled, among whom 170 underwent immunohistochemical analysis of PCIF1 expression in tumor tissues to assess its prognostic significance. Multiplex IHC (mIHC) staining for PCIF1, CD4, CD8, and FOXP3 was conducted on another 75 paired HCC tissues (tumor vs. adjacent non-tumor) to assess PCIF1 levels in ICB responders. In the Hepa 1-6 subcutaneous model, Pcif1-knockout and control tumor-bearing mice received anti-PD-1 injections to evaluate therapeutic efficacy.

Results Compared to adjacent non-tumor tissues, PCIF1 is significantly upregulated in HCC and independently predicts poor patient survival for both TCGA and 245 HCC patients. Elevated PCIF1 in HCC from TCGA correlated with reduced $CD8^+$ T cell infiltration, increased Tregs, and higher TIDE scores (indicating ICB resistance). mIHC categorized the 75 paired HCC cases tissues into three clusters, with the highest PCIF1 cluster (C2) showing the lowest $CD8^+$ T cells and highest Treg cells density, establishing an immune-suppressive microenvironment. Clinically, HCC patients with low PCIF1-expression exhibited superior response to ICB and prolonged progression-free survival (responders vs. non-responders, media PFS: 10.0 months vs. 6.0 months, HR = 0.36, $P < 0.01$) and overall survival (responders vs. non-responders, media OS: 40.0 months vs. 22.3 months, HR = 0.56, $P < 0.001$). In subcutaneous mouse model, Pcif1-knockout tumors exhibited 95.2% growth inhibition after anti-PD-1 therapy, with significantly lower final tumor weight (0.039 ± 0.012g vs. 0.830 ± 0.329g in controls, $P < 0.05$).

Conclusions PCIF1 modulates HCC's immune landscape by regulating $CD8^+$ T cell and Treg infiltration. It serves as a predictive biomarker for ICB response, with higher expression linked to poor prognosis and ICB resistance. Targeting PCIF1 enhances anti-PD-1 therapy efficacy, providing a potential therapeutic strategy for HCC.

P06.13 EXPLORING EPIGENETIC REGULATORS OF PD-L1 VIA CASPEX PROXIMITY LABELING

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10.1136/JITC-2026-ITOC.49

Background Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy by restoring anti-tumor T-cell activity, primarily through blockade of the PD-1/PD-L1 axis.[1] Under physiological conditions, this pathway contributes to immune tolerance; however, tumors exploit PD-1/PD-L1 signaling to evade immune-mediated destruction, resulting in immunotherapy resistance. Therefore, identifying and targeting cancer-specific epigenetic regulators that drive PD-L1 overexpression offers a selective strategy to overcome immune evasion and reshape the immunosuppressive tumor microenvironment.[2] CASPEX is a CRISPR-based proximity labeling approach that enables locus-specific mapping of promoter-associated protein complexes. CASPEX combines catalytically inactive Cas9(d-Cas9) for specific genomic targeting with the peroxidase APEX2, which biotinylates proximal proteins upon hydrogen peroxide activation.[3] Here, we applied CASPEX proximity labeling to a triple-negative breast cancer cell line (TNBC) to reveal novel epigenetic regulators of PD-L1.

Material and Methods The MDA-MB-231 cell line, which expresses high basal PD-L1 expression, was selected for this study. To stably express the CASPEX system, cells were seeded in 6-well plates at a density of 150,000 cells/well and co-transfected with 2.2 μg and 4.4 μg of CASPEX plasmid and piggyBac transposase through Lipofectamine 3000. After CASPEX transfection, puromycin was added to a final concentration of 2 μg/ml, and selection was carried out for 72 hours. Following selection, cells were tested for doxycycline-inducible expression of the CASPEX construct by applying 2 μg/ml for 24 and 48 hours. CASPEX expression was quantified by flow cytometry using the FITC channel and compared to the dox condition GFP expression for two cell lines, which was subsequently identified and reported.

Results Based on flow cytometry results, 24 hours of doxycycline treatment yielded 96% and 88% GFP-positive cells in 2.2 μg and 4.4 μg transfected groups, respectively. After 48 hours, GFP positivity was maintained at 97.35% and 92.77%. This high-efficiency stable expression confirms a homogenous CASPEX-positive population, providing a robust and reproducible foundation for downstream proximity labeling assays.

Conclusions In this study, we successfully generated a stable CASPEX-expressing TNBC cell line with near-complete induction efficiency. This validated platform ensures reliable, promoter-specific profiling of PD-L1–associated protein complexes while minimizing potential noise from non-expressing cells. Future studies will utilize this high-fidelity system to identify epigenetic regulators through proteomic analyses and validate their functional relevance in immune checkpoint control.

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P06.14 REPROGRAMMING CAR-T CELLS TO OVERCOME TUMOR INTRINSIC RESISTANCE IN SOLID TUMORS

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10.1136/JITC-2026-ITOC.50

Background Pancreatic ductal adenocarcinoma (PDAC) remains refractory to immunotherapy despite major advances in the treatment of several solid tumors. This resistance is driven by a hostile tumour microenvironment and reinforced by tumour-intrinsic immune resistance genes (IRGs) that protect cancer cells from T cell–mediated killing, enabling immune evasion and survival. Identifying and therapeutically targeting such intrinsic resistance mechanisms is critical to improving immunotherapy efficacy in PDAC.

Methods High-throughput small interfering RNA (siRNA)–based genetic screens were performed in PDAC cells to identify IRGstumour-intrinsic regulators of immune resistance. Candidate genes were ranked based on enhanced susceptibility to cytotoxic T cell–mediated killing following gene knockdown, quantified using luciferase-based tumour cell viability assays. A kinase anchoring protein 1 (AKAP1) emerged as a top candidate.

AKAP1 function was evaluated by genetic depletion in PDAC cells subjected to immune-mediated stress, including co-culture with cytotoxic T cells or exposure to tumour necrosis factor alpha (TNF alpha). Downstream signalling events were analysed by immunoblotting, focusing on protein kinase A–dependent phosphorylation of Bcl-2-associated death promoter, mitochondrial translocation, and caspase activation. Synthetic peptides designed to disrupt AKAP1 anchoring to the outer mitochondrial membrane were generated, and their activity was validated by assessing mitochondrial localisation and Caspase 9 activation. Findings obtained by genetic depletion were revalidated using peptide-mediated AKAP1 inhibition.

Functional relevance was assessed in patient-derived PDAC organoids exposed to immune-mediated stress. Mitochondrial metabolism under immune challenge was analysed using Seahorse extracellular flux assays, measuring oxygen consumption rate and extracellular acidification rate. Given the broad physiological role of AKAP1, a tumour-restricted targeting strategy was pursued using chimeric antigen receptor T cells capable of delivering AKAP1 inhibitory peptides upon tumour cell recognition. Peptide localisation and transfer were visualised by fluorescence and confocal microscopy.

Results AKAP1 was identified as a mitochondrial immune resistance regulator in PDAC. AKAP1-regulated protein kinase A suppressed apoptosis by phosphorylating the pro-apoptotic protein Bcl-2-associated death promoter, thereby limiting mitochondrial translocation and downstream caspase activation. siRNA-mediated depletion of AKAP1 sensitised PDAC cells to cytotoxic T cells and T cell–derived effector molecules, including TNF alpha. Pharmacological disruption of AKAP1 anchoring using inhibitory peptides lowered the apoptotic threshold of tumour cells and resulted in robust activation of Caspase 9, the key downstream effector of the intrinsic mitochondrial apoptotic pathway. Plasmid-expressed AKAP1 inhibitory peptides demonstrated complete mitochondrial co-localisation, confirming effective engagement of mitochondrial apoptotic signalling.

Conclusion These findings identify AKAP1 as a tumour-intrinsic mitochondrial immune resistance node in PDAC. Targeting AKAP1-mediated survival signalling enhances immune-mediated tumour cell killing and represents a promising strategy to overcome resistance to immunotherapy in pancreatic cancer.

Poster Session 7. Polyspecific Antibody Derivatives

P07.01 TARGETING CANCER ANTIGENS WITH DE NOVO DESIGNED PROTEIN BINDERS AS BISPECIFIC T CELL ENGAGERS

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10.1136/JITC-2026-ITOC.51

Background Nectin-4 is a tumor-associated antigen (TAA), which is overexpressed in a variety of malignancies including urothelial, breast and lung cancer. While targeting Nectin-4 is mainly focused on antibodies or its derivatives in the context of antibody-drug conjugates, we wanted to explore alternative approaches. With recent advances in modern technology of artificial intelligence (AI), predicting protein structures using algorithms like AlphaFold or RoseTTAFold has immensely changed the field of structural biology. Deep-learning pipelines have been developed for designing target-specific or completely de novo protein structures. Therefore, we aimed to implement an AI-assisted pipeline to generate specific protein binders against relevant TAAs like Nectin-4, thereby circumventing long and laborious processes involved in antibody production.

Methods We utilized the generative protein design model RFdiffusion combined with a novel refinement algorithm to create a diverse library of de novo protein binders specific to human Nectin-4. The generated library, comprising thousands of artificial proteins, was screened using a mammalian cell-surface expression system. Functional binders were identified through fluorescence-activated cell sorting and next-generation sequencing. Potential candidates were individually validated with flow cytometry, biochemically characterised via surface plasmon resonance (SPR) spectroscopy and assessed in functional assays.

Results In total, 40 binder candidates were detected from pooled screens and target-specific binding was confirmed. Assessment of binding kinetics revealed high affinities in a low nanomolar range. Additional functionalisation into multivalent formats allowed the application of our binders in flow cytometry assays discriminating Nectin-4 expressing and non-expressing cells. By employing these binders as bispecific T cell engagers (TCEs), we could further demonstrate the activation and T cell-mediated cytotoxicity selectively upon target recognition.

Conclusion Our pipeline proves the feasibility of generating de novo protein binders targeting clinically relevant cancer antigens. By integrating diverse immuno-engineering and computational protein design strategies, we selected and characterized high-affinity clones for translational applications, such as bispecific TCEs. Ultimately, our project advances targeted protein design, supporting the development of customizable therapeutics and diagnostics tailored to patient-specific tumor profiles.

Poster Session 8. Tumor Immunology

P08.01 NOVEL ANTI-PD1 PREDICTIVE SIGNATURE AND FUNCTIONAL DENDRITIC-CELL BIOMARKERS IN MELANOMA IDENTIFIED WITH SYSTEMS IMMUNOLOGY

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10.1136/JITC-2026-ITOC.52

Background Despite the remarkable clinical success of anti-PD-1 immunotherapy in melanoma, identifying functional biomarkers to predict response and guide rational treatment combinations remains a major challenge. Current biomarkers used in clinical practice are largely limited to single-parameter indicators, such as PD-1 expression and $CD8^+$ T cell infiltration. However, the existence of non-responsive patients with high $CD8^+$ T cell infiltration underscores the insufficiency of these markers. Emerging evidence suggests that effective dendritic cell (DC)–$CD8^+$ T cell crosstalk within the tumor microenvironment is critical for therapeutic efficacy, yet no predictive signatures currently capture this key interaction.

Material and Methods To address this limitation, we developed a systems immunology approach to identify immune signatures from whole-transcriptome data across multiple anti-PD-1–treated metastatic melanoma cohorts. Focusing on DC–$CD8^+$ T cell crosstalk, we used CD74 and CD8A as reference biomarkers to select correlating biomarkers, that were incorporated into a logistic regression framework, resulting in the identification of a 15-gene signature, PRIME. Prediction power of PRIME was evaluated in anti-PD-1 pre-treatment samples from multiple transcriptomic metastatic melanoma datasets. These immune signatures also enabled functional biomarker discovery through unsupervised clustering of gene expression, clinical, and functional data. Potential biomarkers were filtered from antigen processing and presentation -related clusters using spatial transcriptomics and single-cell RNA sequencing datasets. The immunogenic functions of selected biomarkers were evaluated in human monocyte-derived dendritic cells (moDCs) by assessing antigen processing efficacy, expression of costimulatory molecules, and alterations in cytokinome upon biomarker gene expression silencing.

Results Our bioinformatic analysis identified 3,359 and 2,902 genes positively and negatively associated with survival of anti-PD-1-treated patients. Of these, 667 genes from the positive group and 129 genes from the negative group showed a significant correlation with DC-CD8+ T cell axis through correlations with CD8A and CD74. Subsequent PRIME model outperformed previously published models in predicting anti-PD-1 response, particularly in tumors with high $CD8^+$ T cell infiltration, reflecting a functional immune state driven by DC–$CD8^+$ T cell interactions. Filtering of potential functional biomarkers highlighted SLAMF7 and TYMP as novel biomarkers associated with DC–$CD8^+$ T cell interactions, based on their expression patterns in dendritic cells and correlation with altered levels of melanoma antigen–specific T cells. Mechanistic studies in moDCs revealed that these biomarkers regulate exogenous antigen processing, expression of costimulatory molecules CD80 and CD40, and secretion of multiple cytokines and chemokines implicated in regulation of DC and T cell functions and migration.

Conclusion In conclusion, these results functionally link the identified biomarkers to key immunological processes underlying anti-PD-1 responses. Together, our findings present a new strategy for predicting anti-PD-1 responses in melanoma and uncover new biomarkers with functional roles that may inform future therapeutic development to enhance clinical efficacy.

P08.02 PD-L1 BLOCKADE PRIMES MEMORY $CD8^+$ T CELLS WITH HIGH CYTOTOXIC POTENTIAL FOR LASTING ANTITUMOR IMMUNITY

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10.1136/JITC-2026-ITOC.53

Background Neoadjuvant anti–PD-L1 therapy is an emerging strategy for the treatment of resectable tumors, in which immune checkpoint blockade is administered prior to surgery. Although this approach has been associated with high pathologic response rates and improved clinical outcomes, the immunological mechanisms underlying these benefits remain incompletely understood.

Methods We employed a mouse model of primary tumor inoculation followed by neoadjuvant anti–PD-L1 treatment and subsequent tumor resection. To assess long-term antitumor immunity, mice were rechallenged with tumor cells after surgery. Antigen-specific $CD8^+$ tumor-infiltrating lymphocytes (TILs) were analyzed following rechallenge, and single-cell RNA sequencing was performed on antigen-specific $CD8^+$ T cells isolated from splenocytes after tumor resection.

Results PD-L1 blockade during the primary tumor phase was sufficient to confer enhanced resistance to tumor rechallenge. Antigen-specific $CD8^+$ TILs from anti–PD-L1–treated mice exhibited augmented effector characteristics following rechallenge. Single-cell transcriptomic analysis revealed the expansion of a distinct antigen-specific $CD8^+$ T cell population characterized by the expression of a specific transcription factor.

This population displayed a memory phenotype and robust cytotoxic potential in response to PD-L1 blockade.

Conclusion These findings demonstrate that neoadjuvant PD-L1 blockade promotes the generation of long-lived, tumor-specific memory $CD8^+$ T cells that are critical for durable antitumor immunity, providing mechanistic insight into the therapeutic benefit of neoadjuvant immune checkpoint inhibition.

P08.03 DRUG SCREENING REVEALS EPIGENETIC REGULATORS OF PD-L1 EXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER

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10.1136/JITC-2026-ITOC.54

Background Immunotherapy has emerged as an effective cancer treatment by reactivating the immune system against tumor cells.[1] However, tumor-intrinsic upregulation of PD-L1 constitutes a major immune-evasion mechanism, suppressing T cell–mediated antitumor immunity.[2 3] Although immune checkpoint blockade with anti–PD-1 or anti–PD-L1 antibodies can restore T cell function, durable clinical responses are achieved in only a subset of patients.[4] These limitations underscore the need to identify upstream regulatory mechanisms that control PD-L1 expression in cancer cells. Emerging evidence suggests that epigenetic regulation plays a crucial role in modulating PD-L1 expression and influencing responses to immunotherapy.[5] Here, we conducted a systematic pharmacological screen using 145 drugs targeting various epigenetic enzymes to identify key chromatin-based regulators of PD-L1 expression in triple-negative breast cancer (TNBC) cells.

Methods A tumor cell line model with high basal PD-L1 expression was selected for this study. MDA-MB-231 cells were seeded in 12-well plates at a density of 50,000 cells/well and treated with 5 μM of the Epigenetic Drug Library (#11076) for 48 hours. Following treatment, cells were fixed with 4% formaldehyde and stained with a cell surface–specific anti–PD-L1 Alexa Fluor 488–conjugated antibody (#25048) diluted 1:200 in 0.5% BSA/PBS buffer. PD-L1 surface expression was quantified by flow cytometry using the FITC channel and compared to vehicle-treated cells. Compounds that significantly modulated PD-L1 surface expression were subsequently identified and reported.

Results Based on the screening results, PFI-4 (BRPF1 inhibitor), CPTH2 (GCN5 inhibitor), SGC-CBP30 (CBP/p300 inhibitor), and Bromosporine (BRD4 inhibitor) significantly reduced PD-L1 surface expression in MDA-MB-231 cells.

Conclusions Our findings highlight tumor-specific epigenetic vulnerabilities that govern immune checkpoint regulation. Importantly, this study identifies multiple epigenetic targets whose pharmacological inhibition may reduce PD-L1 expression in PD-L1–positive tumors and thereby provide a strong rationale for tumor context–specific combination treatment strategies. Future studies will assess the direct binding of the identified epigenetic regulators to the PD-L1 promoter using ChIP, determine their causal role via CRISPR-mediated gene editing, and evaluate the therapeutic potential of epigenetic–immunotherapy combinations in functional immune assays.

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P08.04 EFFICACY AND SAFETY ANALYSIS OF CAMRELIZUMAB COMBINED WITH CHEMOTHERAPY IN THE TREATMENT OF ADVANCED PANCREATIC CANCER

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10.1136/JITC-2026-ITOC.55

Background Advanced pancreatic cancer progresses rapidly and carries an extremely poor prognosis, with chemotherapy providing only limited survival benefits. Although immunotherapy has achieved significant efficacy in many solid tumors, its effectiveness in pancreatic cancer remains limited.

Methods This study was a single-arm, prospective, exploratory trial. Eligibility criteria included patients with histologically or cytologically confirmed advanced pancreatic cancer who had not previously received systemic chemotherapy or who had experienced disease progression after first-line treatment. Enrolled subjects received camrelizumab in combination with chemotherapy (mFOLFIRINOX or AG regimen). The primary endpoint was the objective response rate (ORR).

Results From June 2021 to December 2025, a total of 39 subjects were enrolled in the study. The median age was 58 years (range from 44-77 years), with 61.5% (24/39) male. Among them, 74% (29/39) were first-line treatment subjects and 23% (9/39) were second-line treatment subjects. Metastasis was observed in 74% (29/39) of the subjects, with the liver and lymph nodes being the most common metastatic sites. As of December 30, 2025, tumor response was evaluable in all subjects. The ORR was 28%, and the disease control rate (DCR) was 84%.The median progression-free survival (mPFS) of all subjects was 8.8 months (95% CI, 8.26-9.35). The mPFS was 8.08 months (95% CI, 7.4-8.67) in subjects with pancreatic cancer and multiple-site metastases, and 10.44 months (95% CI, 9.42-11.3) in those without metastases. The median overall survival (mOS) of all subjects was not reached. Most treatment-related adverse events (TRAEs) were of grade 1-3, with no grade 4-5 adverse events reported. The most common TRAEs included anemia (100%, 39/39), lymphopenia (87.2%, 34/39), and leukopenia (71.8%, 28/39).

Conclusion Camrelizumab combined with chemotherapy showed significant efficacy and a favorable safety profile in the treatment of advanced pancreatic cancer, especially in patients with advanced pancreatic cancer and multiple-site metastases. Further large-scale clinical studies are needed to confirm this observation.

P08.05 DEVELOPING AN IN VITRO CUTANEOUS MELANOMA SKIN MODEL TO STUDY THE RESPONSE AND RESISTANCE TO IMMUNOTHERAPY

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10.1136/JITC-2026-ITOC.56

Background Cutaneous melanoma (CM) is the most aggressive form of skin cancer, with an increasing incidence in recent years. Immunotherapy is considered the most promising therapeutic option for late-stage CM. However, resistance occurs in 40-65% of the cases and 20–30% of responding patients develop secondary resistance, mainly due to the immunosuppression imposed by the tumor microenvironment. For this reason, it is essential to investigate the complex interactions between immune cells and tumors.

Methods So far, most of the studies in this context have relied on in vivo models. However, these models present both scientific and ethical limitations. In our lab, we have established an in vitro CM skin model that fully recapitulates skin pathophysiology. Therefore, our objective is now to optimize an immune competent in vitro CM skin model to study the crosstalk between tumor cells and the immune system, and to investigate the mechanisms underlying immunotherapy response and resistance. For this, we developed an in vitro CM skin model composed by a dermis equivalent made with fibroblasts and the extracellular matrix they secrete, and an epidermis equivalent composed by CM cells and keratinocytes that stratify into all the layers of the epidermis. Once the model was fully established, we added to the medium PBMCs previously isolated from healthy donors. To determine the best conditions that allow the immune cells to infiltrate the skin equivalent and migrate toward the CM cells, we tested different immune/CM cell ratios (3:1; 10:1; 30:1) and different incubation times (2, 3 and 4 days). In parallel, as a control condition, we performed the same model but without immune cells. After fixation, the models have been processed for paraffin embedding (for histological analysis with haematoxylin and eosin staining (H&E)) and cryopreservation (for immunofluorescence microscopy analysis).

Results It is possible to recognize by H&E staining the presence of small round cells in the dermis of the model to which PBMCs were added for 4 days at a 30:1 ratio of PBMCs/CM cells, consistent with the morphology of this type of cells. This observation suggests that there was infiltration of PBMCs into the CM skin model. However, since H&E staining is not sufficient to discriminate between PBMCs and the other cell types, we intend to perform immunofluorescence using an antibody that recognizes CD45, a marker that is specifically expressed by PBMCs.

Conclusion The results obtained are promising due to the presence in the dermis of small, rounded cells that are morphologically compatible with PBMCs, suggesting their infiltration in the model. However, immunofluorescence staining is essential to confirm the results. Once established, this model will provide a clinically relevant platform to identify predictive biomarkers of immunotherapy response and novel therapeutic strategies to overcome immunotherapy resistance in CM.

P08.06 NFDI4IMMUNO: A FEDERATED FAIR INFRASTRUCTURE TO ACCELERATE DATA SHARING IN CANCER IMMUNOTHERAPY

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10.1136/JITC-2026-ITOC.57

Background Precision cancer immunotherapy relies heavily on integrating high-dimensional datasets from diverse patient cohorts. However, strict GDPR requirements and data silos often hinder effective multi-center collaboration. Physicians and translational researchers face significant hurdles in accessing and sharing unique immunological data. NFDI4Immuno addresses this gap by establishing a specialized, GDPR-compliant infrastructure within the National Research Data Infrastructure (NFDI) to facilitate the secure open access sharing and analysis of complex immunological datasets in the near future.

Material and Methods The complexity and diversity of immunological data requires robust and standardized research data solutions. NFDI4Immuno addresses this challenge by developing a network of federated institutional repositories specifically designed for the different types of immunological data. The consortium brings together 15 partners (primary research institutions and Universities) across Germany to implement the FAIR (Findable, Accessible, Interoperable, and Reusable) principles across the immunological data landscape. It combines technical developers and operational young and senior experts in the fields, who collaboratively work in a structured, reflecting and constructive way, ensuring that the technical solution aligns directly with the practical needs of data producers and clinical users.

Results We have defined and intensively worked on the definition and effective use of personal resources for a product, which will deliver a working end-to-end workflow. Authenticated users can securely deposit datasets via a standardized API, ensuring immediate discoverability while maintaining strict access controls. They can search for relevant data, and download datasets they have access to. Critical GDPR compliance features have been established. Additionally, the repository delivers user support structures in form of a helpdesk system and training material. The technical architecture of NFDI4Immuno includes developed software stacks and automated analysis pipelines. The initial focus is on Adaptive Immune Receptor Repertoire Sequencing (AIRR-seq) and cytometry data, whereas future plans include expanding to include other immunological data classes like reactivity and immunopeptidomics. The federated repository infrastructure enables researchers to deposit, discover and access high quality immunological data while maintaining appropriate access management and provenance tracking.

At its core, NFDI4Immuno works towards seamless interoperability with other life sciences NFDI consortia, particularly GHGA, NFDI4Health, NFDI4Microbiota and NFDI4BIOIMAGE. Additional efforts focus on mapping to and integrating with existing external resources such as the AIRR Data Com-

mons, creating harmonized data access and standardized data representations. This interoperability strategy enables cross-domain data discovery and reuse, facilitating novel insights at the intersection of immunology and related disciplines.

Conclusion NFDI4Immuno will offer a practical, robust solution for the cancer immunology community, moving beyond theoretical concepts to a functioning service. By providing a secure environment for data federation, it empowers researchers to leverage collective data resources. This infrastructure not only fulfills 'Open Science' mandates but actively accelerates innovation by making valuable clinical data accessible for translational research.

P08.07 ABSTRACT WITHDRAWN

P08.08 MODULATION OF CD30 RECEPTOR SHEDDING IN THE PRESENCE OF EDTA ON K562 CELLS PRETREATED WITH TNF

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10.1136/JITC-2026-ITOC.58

Background The TNF/TNF receptor system plays an important role in signal transduction for the induction of apoptosis and necrosis. TNF receptors are a complex group of proteins consisting of several families of receptors and are activated by the cytokine TNFα. In this work, the role of EDTA as an inhibitor of the TACE signaling pathway on the expression of the surface CD30 antigen on K-562 cells after apoptosis induction was investigated.

Material and Method K562 cells were treated with and without the presence of the cytokine TNF-α at a concentration of 1000 pg/ml for 24 h with and without the presence of EDTA in an incubator with 5% CO2. After treatment, the expression of the CD30 molecule was examined on a BD, Fax verse (USA) flow cytometer, the degree of cell death was examined using LDH release, while CD30 in the supernatant of cell cultures was determined using the Western blot technique.

Results The results showed that TNF-a led to a statistically significant decrease in the expression of the CD30 molecule on the membrane of K562 cells ($p < 0.05$, Mann Whitey, U-test), to a statistically significant increase in LDH inducing cell death. Examination of the expression of CD30 on the membrane of K562 cells after the addition of EDTA, which is an inhibitor of the TACE enzyme responsible for receptor cleavage, resulted in a partially reversible decrease in expression and a slight increase in the value by 10% compared to TNF-α without the presence of EDTA.

Conclusion It has been shown that EDTA as an inhibitor of the enzyme that blocks signal transmission through the TNF family of receptors can reduce the effects on cells, but the results are dose-dependent and further studies are needed to show the blockade depending on the receptor in order to achieve a stronger effect. Further studies should be directed to the presence of parts of the molecule in the supernatant of cell cultures in order to prove parts of extracellular receptor fragments.

P08.09 A ONE-SHOT MULTIPLEX IMMUNOFLUORESCENCE PLATFORM FOR SPATIAL ANALYSIS OF HETEROGENEITY IN VASCULAR IMMUNE NICHES IN CLEAR CELL RENAL CELL CARCINOMA

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10.1136/JITC-2026-ITOC.59

Introduction Clear cell renal cell carcinoma (ccRCC) is characterized by a highly heterogeneous tumor vasculature, which varies in density and architecture and is associated with prognosis and response to anti-angiogenic therapy and immune checkpoint inhibition.[1 2] Despite its clinical relevance, the molecular basis of vascular heterogeneity in ccRCC remains poorly understood. Conventional immunohistochemistry and vessel density measurements do not cover the full complexity of endothelial phenotypes and their spatial interactions with tumor and immune cells.[3–5] This study aims to enable extended spatial characterization of endothelial subtypes in ccRCC using high-throughput multiplex immunofluorescence combined with computational image analysis.

Material and Methods An optimized one-shot multiplex immunofluorescence protocol for formalin-fixed paraffin-embedded (FFPE) tissues was established, enabling simultaneous detection of up to nine markers in a single staining round. The approach combines antibody-oligonucleotide conjugation and conventional secondary antibody staining to maximize sensitivity. Marker panels include endothelial, tumor, immune, stromal, and proliferation markers relevant to the vascular microenvironment in ccRCC. Whole-slide imaging was performed using widefield fluorescence microscopy for rapid acquisition of large tissue areas. A fully automated image analysis pipeline was developed, including denoising, spectral unmixing, background subtraction, tissue segmentation, and single-cell quantification. Vascular architectures were classified using pattern recognition algorithms.

Results The staining protocol yielded reproducible, high signal-to-noise detection of all markers across full-slide FFPE sections with minimal autofluorescence and preserved tissue morphology. The one-shot workflow enabled staining and imaging of multiple whole-slide samples within 48 hours, substantially increasing throughput compared with sequential multiplexing approaches. The computational pipeline reliably separated spectrally overlapping signals, enabled robust tissue and cell segmentation, and supported quantitative spatial analysis at single-cell resolution, demonstrating the technical suitability of the platform for large-scale spatial profiling.

Conclusion This work establishes a scalable, high-throughput multiplex immunofluorescence and analysis workflow optimized for FFPE ccRCC tissues. The platform provides a robust technical foundation for future systematic investigation of endothelial heterogeneity and tumor-microenvironment interactions and is readily extendable to larger cohorts and additional cancer types.

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P08.10 PATHOLOGICAL IMMUNE AGGREGATES DRIVEN BY HMGB1 LIMIT ANTITUMOR IMMUNITY IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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10.1136/JITC-2026-ITOC.60

Background B cells are frequently enriched in the peritumoral region of esophageal squamous cell carcinoma (ESCC). Our previous work showed that ESCC-derived HMGB1 drives peritumoral B-cell proliferation and proangiogenic phenotypes. However, the spatial organization and functional impact of peritumoral immune hotspots remain poorly understood. These clusters, positioned near tumor islands, may create localized immunosuppressive architecture distinct from the tumor core. Understanding how HMGB1 influences hotspot formation is critical for improving immunotherapy strategies.

Methods Multiplex immunofluorescence and spatial analysis (QuPath) were performed on 43 ESCC specimens. Tumors were classified as B-rich if immune nests contained ≥60,000 μm^2 area, ≥700 total cells, and ≥350 $CD20^+$ cells. HMGB1 expression was quantified in $PanCK^+$ tumor cells. To assess immune architecture, a B-cell spatial score was calculated using automated algorithms for each $CD20^+$ B cell as the ratio of its distance to the nearest $PanCK^+$ tumor cell (distance A) over its distance to the nearest B cell (distance B). A higher score indicates that B cells are more intermixed with other B cells (i.e., forming stronger clusters away from tumor nests), whereas a lower score reflects closer proximity to tumor cells. Functional assays included B-cell aggregation, optical tweezer-based adhesion force measurements, and transwell co-culture of $CD20^+$ B cells and $CD8^+$ T cells under HMGB1 gain-of-function (GOF) or knockout (LOF) conditions.

Results Of 43 specimens, 6 were excluded due to poor staining quality, leaving 37 evaluable cases; 17 tumors were classified as B-rich and 20 as non-B-rich. B-rich tumors exhibited significantly higher B-cell spatial scores compared to non-B-rich tumors (Chi-square test, $p=0.0309$), indicating B cells in B-rich tumors form dense clusters away from tumor cells rather than integrating into tumor nests. HMGB1-high tumors correlated with shorter CD20–CD20 distances ($r = -0.3394$), suggesting HMGB1 promotes B-cell clustering. In vitro, a transwell system was used to mimic spatial positioning, where $CD20^+$ B cells were either retained in the upper chamber (UC; farther from tumor cells) or allowed to migrate to the lower chamber (LC; direct contact with tumor cells). HMGB1 GOF increased B-cell aggregation and adhesion strength. Furthermore, multiplex imaging demonstrated $CD8^+$ T cells preferentially localized to B-rich areas, and coculture revealed reduced tumor cell death when B cells were paired with $CD8^+$ T cells under GOF conditions, whereas HMGB1 LOF reversed this trend. These findings suggest that HMGB1-driven B-cell aggregates may trap $CD8^+$ T cells away from tumor cells, limiting cytotoxicity.

Conclusion HMGB1 promotes the formation of dense B-cell aggregates at peritumoral immune hotspots, which sequester functional $CD8^+$ T cells away from tumor cells and impair cytotoxicity. By reshaping immune architecture, HMGB1 emerges as a key regulator of spatial immune dysfunction and a promising therapeutic target for dismantling pathological aggregates to restore effective antitumor immunity.

P08.11 INTEGRATIVE MOLECULAR AND SPATIAL ANALYSIS OF ENDOTHELIAL CELL HETEROGENEITY IN CLEAR CELL RENAL CELL CARCINOMA

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10.1136/JITC-2026-ITOC.61

Background Accounting for 80% of all cases, the clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of renal cell carcinoma. ccRCC exhibits resistance to common chemotherapies and a highly variable response to treatment with angiogenesis inhibitors, immune checkpoint blockade or a combination thereof. This, and its high tendency to metastasize necessitate advancements in therapy options. As a highly vascularized tumor type, ccRCC has been shown to exhibit different inter- and intratumoral vascularization patterns, high- and low-branching, which have been linked to therapy response and prognosis.[1–4] As endothelial cells are characterized by a vast phenotypic and functional heterogeneity, we aim to elucidate the molecular heterogeneity of endothelial cells in ccRCC and their potential role in primary or acquired resistance by identifying a possible molecular correlate to the tumors vascular architecture.

Materials and Methods Matched and unmatched tumor and non-neoplastic tissue were collected from patients who underwent partial or total nephrectomies between 2022 and 2024 in the Department of Urology at the University Hospital Bonn and gave informed consent for biobanking. A protocol for the enrichment of isolated endothelial cells was established for single nuclei RNA sequencing as well as cultivation of the patient-derived endothelial cells. snRNA-sequencing was performed to identify a molecular correlate to the histology while tube-formation experiments were performed to characterize patient-derived endothelial cells‘ behavior in vitro. Xenium spatial transcriptomics experiments were performed to explore the previous findings in a spatial context.

Results Six distinct endothelial cell clusters as well as two pericyte clusters were identified, including an endothelial cell cluster highly enriched in non-neoplastic tissue. Two of the identified clusters showed enrichment in samples with predominantly low-branching vascular architecture. Differences in tube-formation-behavior between endothelial cells isolated from the dif-

ferent patterns were observed. Using a custom-panel built from DEG identified in the snRNA-seq dataset, vessels corresponding to the disctint EC clusters were identified.
Conclusions The results of this work indicate a link between molecular endothelial cell heterogeneity and vascular patterns in ccRCC and suggest a functional heterogeneity linked to the vascular architecture.

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P08.12 TBX21 AS A TRANSCRIPTION FACTOR ASSOCIATED WITH IMMUNOTHERAPY RESPONSE IN COLORECTAL CANCER

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10.1136/JITC-2026-ITOC.62

Background Immune checkpoint blockade has significantly improved outcomes in a subset of colorectal cancer (CRC) patients; however, reliable biomarkers for predicting therapeutic response remain limited. This study aimed to identify transcription factors associated with immunotherapy response in CRC and to explore their prognostic value across multiple cancer types.
Methods Single-cell RNA sequencing (scRNA-seq) datasets of colorectal cancer patients receiving immunotherapy were obtained from the Gene Expression Omnibus (GEO) database. Cell type annotation and transcription factor activity analysis were performed to identify differentially regulated transcription factors among immune cell populations. To further evaluate the clinical relevance of key transcription factors, Kaplan–Meier survival analyses were performed to investigate the association between transcription factor expression and patient overall survival.
Results Single-cell analysis revealed significant transcriptional reprogramming of immune cells following immunotherapy, particularly within the T-cell compartment. Among the identified transcription factors, TBX21 was significantly upregulated in T cells after treatment compared with pre-treatment samples. Elevated TBX21 expression was associated with enhanced immune activation signatures, suggesting a role in effective antitumor immune responses. Survival analyses indicated that high TBX21 expression was associated with improved overall survival in patients with colon adenocarcinoma (COAD), kidney renal clear cell carcinoma (KIRC), and skin cutaneous melanoma (SKCM).
Conclusion Our study identifies TBX21 as a key transcription factor associated with immunotherapy response in colorectal cancer. The upregulation of TBX21 in T cells following treatment and its favorable prognostic value in multiple tumor types suggest that TBX21 may serve as a potential biomarker for predicting immunotherapy efficacy.

P08.13 THE MEVALONATE PATHWAY FUELS THE IMMUNOGENIC RESPONSE TO CANCER IN PLASMACYTOID DENDRITIC CELLS

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10.1136/JITC-2026-ITOC.63

Background Cancer immunotherapy has the potential to treat a wide range of human cancers. Plasmacytoid dendritic cells (pDC) are a subset of type-I interferon (IFN-I) producing dendritic cells, and we have previously shown that they can elicit robust anti-tumor immune responses upon activation with Toll-like receptor 7 (TLR7) agonists such as Imiquimod (IMQ). In contrast, in the absence of activating signals, pDCs often acquire pro-tumorigenic functions. The aim of this study is to define the molecular switches that determine the pro- or anti-tumor activity of pDCs in tumors of differing immunogenicity.
Methods Here, we combined genetically engineered mouse models with preclinical tumor models, next-generation sequencing, and immune profiling assays to uncover a previously unappreciated role for pDCs in immunogenic anti-tumor responses.
Results To assess the role of pDCs in tumors of differing immunogenicity, we implanted a pair of progressor and regressor YUMM1.7 melanoma cell lines into Bdca2-DTR mice. Depletion of pDCs in the progressor tumor model had no detectable effect on tumor growth. By contrast, pDC depletion abrogated tumor rejection in immune-inflamed regressing tumors. Transcriptional profiling of pDCs isolated from progressor and regressor tumors revealed a distinct metabolic signature in pDCs from immune-inflamed tumors, characterized by engagement of the cholesterol biosynthesis (mevalonate) pathway. Consistently, pharmacological inhibition of the mevalonate pathway impaired pDC maturation upon TLR7 agonist stimulation, demonstrating that cholesterol uptake and de novo synthesis are required in activated pDCs.
Conclusion pDCs are required for tumor regression in immune-inflamed tumors and are distinguished by a metabolic program centered on the mevalonate pathway. These findings identify metabolic regulation as a key determinant of pDC-mediated anti-tumor immunity and provide a framework for therapeutic strategies aimed at enhancing pDC function in cancer.

P08.14 UNDERSTANDING TIME-RESOLVED IMMUNE LANDSCAPE TO INDUCT A COMBINATORIAL PHOTOTHERMAL-IMMUNOTHERAPY APPROACH FOR TREATMENT OF ADVANCED-STAGE CANCER

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10.1136/JITC-2026-ITOC.64

Background Photothermal therapy (PTT) is a precise solid tumor treatment modality that causes rapid tumor ablation through localized hyperthermia. Beyond direct cytotoxicity, PTT induces immunogenic cell death by releasing damage-associated molecular patterns that prime systemic anti-tumor immunity. However, PTT-induced immune activation exhibits

temporal constraints due to compensatory immunosuppressive mechanisms. This study investigates the temporal dynamics of PTT-induced immune responses in the tumor microenvironment (TME) and systemically to identify a critical post-treatment window when the post-PTT activation phase transitions to the suppressive phase. Leveraging these insights, we propose a rational combinatorial strategy along with PTT during the therapeutic window to sustain anti-tumor immunity and improve outcomes in advanced-stage malignancies.

Methods Photothermal therapy was performed in an orthotopic Balb/c mouse model via intratumoral Au-SLN administration followed by 5-minute NIR laser irradiation (750nm, 3.3W/cm^2). Tumor progression was longitudinally monitored using bioluminescence imaging. Comprehensive flow cytometry and histology-based immunophenotypic assessment of infiltrating immune cells in TME and systemic inflammatory mediators was conducted after tumor and serum sample collection, respectively, at predetermined intervals of 24 hours, 4 days, and 11 days post-PTT. To evaluate PTT-induced systemic anti-tumor immunity, a bilateral tumor model was employed wherein the untreated tumor served as the distant site for assessing abscopal responses mediated by photothermal therapy of the primary site.

Results Single-dose photothermal therapy induced rapid tumor ablation, with significant bioluminescence reduction ($p<0.05$) within 24 hours and marked architectural disruption at the laser-targeted site. Post-PTT, early TME revealed robust anti-tumor immune activation, with enhanced infiltration of pro-inflammatory M1 macrophages ($p=0.0068$), activated dendritic cells ($p=0.03$), CD4+ helper T cells ($p=0.0084$), and CD8+ cytotoxic T cells ($p<0.05$). Systemic immune activation was confirmed through elevated serum levels of Th1-associated cytokines, particularly IL-12, and chemokines. However, by day 11 post-treatment, immune cell infiltration declined significantly ($p<0.05$), accompanied by a reduction in systemic Th1/Th2 cytokine ratios, indicating immunosuppressive reprogramming and emergence of immune evasion mechanisms. Nevertheless, PTT demonstrated systemic anti-tumor potential in bilateral tumor models, delaying metastatic progression by 14 days and extending survival by approximately 20 days versus untreated controls. The observed biphasic immune response from the initial activation phase to late immunosuppression identified a critical therapeutic window (days 4-11 post-PTT). These dynamics provide a strong rationale for combinatorial immunotherapy employing checkpoint inhibitors (anti-PD-L1) and macrophage-targeting antibodies (anti-CD47) during this period to sustain T cell function, maintain M1 polarization, prevent immunosuppressive reprogramming, and amplify systemic abscopal effects for durable anti-tumor responses.

Conclusion PTT elicits potent anti-tumor effects through thermal ablation and immune activation, generating systemic abscopal immunity. However, a temporal decline in immune activation requires intervention. The identified 4-11-day therapeutic window provides a rational framework for integrating combinatorial treatment modalities, positioning PTT as an effective immunotherapeutic modality for sustained anti-tumour responses.

P08.15 INSECT JUVENILE HORMONE III IS A POTENTIAL NOVEL AND HIGHLY EFFICIENT AGENT FOR IMMUNOTHERAPY OF CANCER

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10.1136/JITC-2026-ITOC.65

Background and Aims Immunotherapy of cancer is a rapidly developing and very promising field of biomedical science and clinical medicine. However, immunotherapy sometimes fails due to monoclonal antibodies used for it do not reach tumours as expected or cause autoimmune side effects. As such, there is a highly unmet need of designing small molecular weight drugs which can downregulate cancer immune evasion machinery and/or sensitivity of cytotoxic T cells to immune checkpoints used by cancer cells to suppress anti-tumour immunity. This would allow the immune system to attack and eradicate cancer cells and malignant tumours. In this work we studied naturally occurring small molecular weight sesquiterpenoid insect juvenile hormone III as a potential agent for highly efficient immunotherapy of cancer.

Methods A wide range of techniques including tissue culture and co-cultures, Western blot analysis, on-cell Western, ELISA, co-immunoprecipitation, ChIP, biochemical assays and synchrotron radiation circular dichroism spectroscopy were employed.

Results Here we report for the very first time that Juvenile hormone III (insect hormone), a small molecular weight compound, which is not produced in humans, impacts human cytotoxic T cells, rendering them resistant to cancer immune evasion machinery and enabling them to kill cancer cells. This is achieved by downregulation of expression of immune checkpoint receptors such as PD-1 (Programmed Death-1), VISTA (V-domain Ig-Containing Suppressor of T Cell Activation) and Tim-3 (T cell immunoglobulin and mucin domain containing protein 3). Juvenile hormone III also demonstrated the ability to downregulate expressions of immune checkpoint proteins, such as galectin-9, VISTA and indoleamine-2,3-dioxygenase 1 (IDO1), thus suppressing cancer immune evasion machinery. We have also discovered the biochemical mechanism underlying the ability of juvenile hormone III to downregulate expressions of the immune checkpoint proteins and receptors mentioned above.

Conclusions Juvenile hormone III has a strong potential to support anti-cancer immunity and attenuate activity of cancer immune evasion machinery.

P08.16 ENHANCING THE IMMUNOGENICITY OF ESOPHAGOGASTRIC ADENOCARCINOMA BY RESTORING ANTIGEN PRESENTATION AND TUMOR-ASSOCIATED ANTIGENS USING EPIGENETIC DRUGS

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10.1136/JITC-2026-ITOC.66

Background Immune checkpoint inhibitors (ICIs) have expanded treatment options for esophagogastric adenocarcinoma (EGA), but primary and secondary resistance limit durable benefit to a subset of patients. Defective antigen processing and presentation is a key mechanism of immune escape and remains poorly understood in EGA. Genes involved in the antigen processing and presentation machinery (APM) are regulated by epigenetic and inflammatory pathways, which are likely susceptible to therapeutic modulation.

Methods Genomic and transcriptomic alterations in APM pathways were analyzed in 43 EGA tumors and validated in an independent TCGA cohort (n = 419). Protein expression of 10 key APM components was assessed by immunohistochemistry (n = 123). EGA cell lines were treated with epigenetic and pro-inflammatory agents, and effects on tumor-associated antigen (TAA) expression, APM component expression, apoptosis, and the immunogenic peptide repertoire were evaluated by flow cytometry and immunopeptidomics. T-cell abundance (immune score) and tertiary lymphoid structures (TLS) in primary tumors were assessed as surrogates of immunogenicity.

Results Although antigen processing and presentation pathways were globally upregulated in tumor tissue compared with patient-matched normal tissue, marked inter-patient heterogeneity and tumor-specific APM dysregulation were observed. APM expression correlated with DNA methylation and interferon-γ signatures, suggesting epigenetic and inflammatory regulation of the APM. Pharmacological modulation with epigenetic and pro-inflammatory drugs restored APM and TAA expression and increased the abundance of potentially immunogenic peptides. Dysregulation of APM-associated molecules was associated with reduced immune scores and TLS abundance.

Conclusion APM dysfunction represents a frequent and therapeutically druggable mechanism of immune escape in EGA. Epigenetic and pro-inflammatory strategies can restore antigen presentation, enhance tumor immunogenicity, and support combination approaches with immunotherapy to overcome resistance.

M.v.B-B: received honoraria for advisory board participation and invited talks from BMS, and financial support for research projects from Astellas, Roche, and MSD. H.A.S: received financial support for research projects from AstraZeneca, unrelated to the content of this project. All other authors declare no conflicts of interest relevant to the content of this project.

P08.17 ABSTRACT WITHDRAWN

P08.18 A SINGLE-CELL AND MACHINE LEARNING-DERIVED PROGNOSTIC SIGNATURE OF CD8⁺ EXHAUSTED T CELLS FOR COLORECTAL CANCER LIVER METASTASIS

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10.1136/JITC-2026-ITOC.67

The tumor microenvironment (TME) is pivotal in the progression and treatment resistance of colorectal cancer liver metastasis (CRLM). The role of $CD8^+$ exhausted T cells ($CD8^+$ Tex) in CRLM remains incompletely understood. This study aimed to construct a single-cell atlas of CRLM to decipher the cellular interactions of $CD8^+$ Tex and develop a prognostic model for clinical application.

We integrated single-cell and bulk transcriptomic data from GEO and TCGA. Using Seurat and CellChat, we characterized the cellular composition and communication in CRLM TME. Metastasis-associated differentially expressed genes were identified by comparing $CD8^+$ Tex from liver metastases and primary tumors. A prognostic signature was built via LASSO-Cox regression and validated in CRC cell lines using RT-qPCR.

The CRLM single-cell atlas revealed marked enrichment of $CD8^+$ Tex in liver metastases. CellChat analysis indicated active cancer-associated fibroblasts (CAFs)–$CD8^+$ Tex crosstalk mediated by collagen signaling. A six-gene prognostic signature (FKBP5, CMC1, NDUFC1, TMSB10, SERF2, SNW1) was established and validated, showing consistent predictive power. Differential gene expression was confirmed in CRC cells. A nomogram combining risk score and clinical features was developed to facilitate prognosis assessment and personalized treatment.

Our study elucidates an immunosuppressive CRLM microenvironment driven by $CD8^+$ Tex–CAFs interactions and presents a clinically applicable $CD8^+$ Tex–based prognostic signature. These insights enhance the understanding of immune evasion in CRLM and offer a potential tool for prognosis and targeted therapy development.

P08.19 APPLICATION OF SDC2 AND SEPTIN9 GENE METHYLATION DETECTION IN COLORECTAL CANCER DIAGNOSIS AND IMMUNOTHERAPY EFFICACY ASSESSMENT

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10.1136/JITC-2026-ITOC.68

SDC2 gene encodes the transmembrane proteoglycan molecule Syndecan-2. Syndecan-2 protein affects the proliferation, migration and invasion of colorectal cancer (CRC) cells by participating in the regulation of cell adhesion, tissue differentiation and angiogenesis. Plasma methylated Septin9 gene has been shown to be a sensitive and specific biomarker for the

detection of CRC, which is involved in apoptosis, pseudopod projection, tumor cell migration and invasion. The aim of this study is to validate the value of SDC2 and Septin9 gene methylation detection in the diagnosis and immunotherapy efficacy evaluation of colorectal cancer.

A total of 102 patients were included in the study. Real-time PCR was used to detect the methylation levels of SDC2 gene in feces and Septin9 gene in blood. The clinical diagnostic accuracy of SDC2 kit was evaluated in patients with gastrointestinal benign lesions, gastrointestinal tumors and healthy subjects. Combined detection of SDC2 and Septin9 can improve the detection rate of CRC. The SDC2m levels of 26 patients with partial remission or stable disease after immunotherapy and 32 patients with complete remission after radical surgery were analyzed.

For eight healthy subjects with negative colonoscopies, the true negative rate was 100%. Data from 72 patients with CRC were evaluated, revealing a sensitivity of 89.1% (41/46; 95% CI: 0.798-0.985) for untreated CRC. In patients who had received immunotherapy and showed partial response or stable disease, the sensitivity of SDC2 was 46.2% (12/26; 95% CI: 0.256-0.667). The sensitivity of Septin9 for CRC detection was 88.2% (15/17; 95% CI: 0.712-1.053). By combining two tests, 94.1% (16/17; 95% CI: 0.816-1.066) of CRC cases could be detected. Sensitivity was 89.1% in patients with untreated CRC and 46.2% in those with partial response or stable disease after immunotherapy. There was a significant difference in the positive rate of SDC2m between the two groups ($P < 0.001$). Among the 32 patients with colorectal cancer who underwent radical surgery, 93.8% (30/32) of the colorectal cancer patients who were initially positive for SDC2m were negative.

Detection of SDC2m in untreated CRC patients has high sensitivity and has the potential to become a non-invasive diagnostic tool for CRC. Combination of Septin9 and fecal SDC2 could improve the detection rate of SDC2 in non-dominant population.The methylation level of SDC2 may be helpful for postoperative follow-up, prediction of recurrence and the efficacy of immunotherapy after radical surgery for CRC.

P08.20 HETEROGENEOUS LONG-TERM T CELL FITNESS FOLLOWING CAR-T THERAPY IN DLBCL AND MULTIPLE MYELOMA

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10.1136/JITC-2026-ITOC.69

Background Chimeric antigen receptor (CAR) T cell therapy is highly effective in relapsed or refractory B cell malignancies, yet long-term outcomes remain heterogeneous, with frequent late relapses. Early post-infusion parameters are strongly influenced by lymphodepletion and antigen burden and therefore provide limited insight into durable immune control. In contrast, evaluation of T cell fitness one year after CAR-T therapy reflects long-term immune adaptation following sustained proliferative and antigenic stress and may reveal mechanisms underlying relapse and durable remission.

Methods Serum and peripheral blood mononuclear cells (PBMCs) were obtained from patients with diffuse large B cell lymphoma (DLBCL) and multiple myeloma (MM) one year after CAR-T cell therapy, as well as from healthy donors (HD). T cell counts, phenotype, and activation were assessed after overnight thawing. T cell activation and cytokine release were evaluated using GFP^+ JeKo-1 cells in the presence of Glofitamab. Long-term T cell function was assessed in a co-culture system with repeated tumor rechallenge. T cell phenotype, proliferation, exhaustion, and residual tumor cells were analyzed by flow cytometry.

Results Serum cytokine profiles were largely comparable between DLBCL and MM patients but differed markedly from those of healthy donors (HD), indicating a shared inflammatory state in both patient cohorts. Compared with MM and HD, DLBCL patients exhibited a pronounced reduction in naïve T cells in both $CD4^+$ and $CD8^+$ compartments (HD: 47.257% ± 14.803, DLBCL: 19.814% ± 19.106, MM: 24.672% ± 21.127), accompanied by an increased frequency of terminally differentiated effector memory (TEMRA) T cells (HD: 16.021% ±4.753, DLBCL: 40.315% ± 25.129, MM: 36.692% ± 13.207), and elevated CD69 expression on $CD8^+$ T cells (HD: 18.423% ±12.484, DLBCL: 84.32% ± 12.28, MM: 69.942% ± 18.512). Although T cells from DLBCL patients displayed increased interferon-α (IFN-α) expression in a 12-hour cytotoxicity assay (HD:39.171% ± 10.662, DLBCL: 58.044% ± 18.821, MM: 56.667% ± 13.576), their functional capacity was inferior to that of T cells from HD and MM. Both DLBCL and MM T cells exhibited limited proliferative capacity during co-culture, with markedly reduced peak expansion (T cell peak number/μl: HD:6068.971 ± 1513.484, DLBCL: 514.567 ± 415.767, MM: 1042.596 ± 1396.375), consistent with decreased IL-2 expression. Patient cohorts showed increased exhausted T cell subsets and reduced naïve T cell frequencies compared with HD. Notably, T cells from DLBCL patients demonstrated the poorest long-term cytotoxicity, followed by MM, as evidenced by fewer effective rechallenge cycles and increased residual tumor burden.

Conclusion Despite achieving clinical remission, patients with DLBCL and MM exhibit persistent T cell dysfunction up to one year after CAR-T cell therapy, marked by reduced immune fitness, sustained inflammation, and depletion of naïve T cells, suggesting durable immune remodeling beyond tumor clearance that may contribute to heterogeneous long-term remission and late complications.

P08.21 ABSTRACT WITHDRAWN

Poster Session 9. Young Researcher Session

P09.01 BLINATUMUMAB RESISTANCE IN PAEDIATRIC RELAPSED ACUTE LYMPHOBLASTIC LEUKAEMIA IS INTRINSICALLY DRIVEN BYLEUKAEMIA AGGRESSIVENESS ESPECIALLY RESISTANCE TO APOPTOSIS

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10.1136/JITC-2026-ITOC.70

Background Blinatumomab is a bispecific T-cell engager that links $CD3^+$ T cells with $CD19^+$ B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) blasts. It engages patient T cells in vivo without prior genetic modification or lymphodepletion. In children with relapsed BCP-ALL, blinatumomab achieves response rates of 38–60% (Cheng & Liu, 2025), while exhibiting lower toxicity and greater availability than CAR-T therapy. Several resistance mechanisms have been proposed and investigated in vitro or in small cohorts. These mechanisms may be intrinsic to the leukaemia or driven by extrinsic factors such as T-cell dysfunction or the tumor microenvironment (Zhao et al., 2020). In this study, we investigate potential intrinsic mechanisms of blinatumomab resistance in a paediatric cohort with relapsed of BCP ALL.

Methods We performed a retrospective analysis of RNA sequencing data obtained at relapse diagnosis, together with minimal residual disease (MRD) monitoring, from bone marrow samples of 44 paediatric patients diagnosed with relapsed BCP-ALL between 2019 and 2023. All patients received blinatumomab either as part of consolidation therapy or as a bridge to hematopoietic stem cell transplantation (HSCT) and had a detectable MRD prior to blinatumomab. Response to blinatumomab was defined by MRD clearance (< 10e-4 or negative) at the end of the treatment cycle. Patients were stratified into good responders (GR, n = 27) and non-good responders (NGR, n = 17). RNA sequencing and MRD monitoring were performed as part of routine diagnostics. Data were analysed integratively using appropriate bioinformatic and statistical methods.

Results Differential gene expression, gene set enrichment, and gene ontology analyses identified a transcriptional profile enriched for genes involved in cell cycle regulation and DNA repair. Adjustment for blast percentage derived from flow cytometry demonstrated that these signatures were associated with leukaemic blasts. In addition, a mitochondria complex I NDUFS6–CD147 interaction was found to be differentially elevated in NGR patients, consistent with enhanced apoptosis resistance and cell survival. Genes belonging to the inhibitor of apoptosis protein (IAP) family, as well as SERPINB9 and its associated genes, were upregulated in NGR compared to GR. Analysis of CD19 alternative splicing at relapse diagnosis, including intron 2 retention and intraexonic splicing of exon 2 (CD19 ex2part), revealed no significant differences between the two response groups.

Conclusion Leukemia-intrinsic biological features, particularly increased proliferative capacity and apoptosis resistance at relapse diagnosis, are associated with poor response to blinatumomab. In contrast, target-specific adaptations such as CD19 downregulation and alternative splicing appear to emerge during therapy, reflecting treatment-driven tumour evolution as seen in other studies (Zhao et al., 2020). These findings highlight potential diagnostic markers for early prediction of blinatumomab response and support the rationale for adjuvant therapeutic strategies targeting leukaemia biology to overcome resistance.

P09.02 ORAL ADMINISTRATION OF A TGF-B-ENRICHED POLYMERIC DIET MITIGATES GASTROINTESTINAL ACUTE GVHD

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10.1136/JITC-2026-ITOC.71

Background Gastrointestinal acute graft-versus-host disease (GI-aGVHD) is a major contributor to non-relapse mortality after allogeneic hematopoietic cell transplantation (allo-HCT). Standard immunosuppressive therapies risk compromising the beneficial graft-versus-leukemia effect. Nutritional interventions offer a non-immunosuppressive, tissue-protective adjunct. However, mechanistic preclinical evaluations of clinically administered nutritional formulas in aGVHD remain scarce. Addressing this gap, we evaluated whether Modulen® IBD (a casein-based formula enriched with transforming growth factor-beta (TGF-β)) could mitigate GI-aGVHD.

Methods and Results Based on the metabolic signatures of aGVHD reported by Michonneau et al. (Nat. Commun., 2019), we assessed the effects of Modulen® IBD in a pilot clinical study. Allo-HCT recipients (n = 3 to 4) received daily prophylactic Modulen® IBD (1.5 kcal/ml), from the start of conditioning regimen until onset of conditioning-related toxicity. We observed increased levels of anti-inflammatory long-chain (LC) FAs like oleic and eicosatrienoic acids in the serum, as well as elevated kynurenic acid, a microbiota-derived aryl hydrocarbon receptor ligand implicated in intestinal barrier protection. These findings suggest a systemic metabolic shift toward reduced inflammation.

In parallel, we employed a well-established major histocompatibility complex-mismatched murine allo-HCT model (BALB/c → C57BL/6) to validate and expand upon the immune, microbial, and metabolic mechanisms of our clinical study. We administered Modulen® IBD or vehicle orally daily from day 0 to day 14 post-transplant (1kcal/ml). Modulen® IBD treatment significantly reduced histological aGVHD scores in the colon, small intestine and liver of mice. Kaplan-Meier analysis indicated improved survival. Similar to patients, LC-MS analysis of serum from treated mice confirmed increased levels of oleic, linoleic and eicosatrienoic acids. Additionally, there was a reduction in pro-inflammatory LCFAs (palmitic, stearic, and arachidonic acids) and GVHD-associated amino acids (arginine, ornithine, leucine, and aspartate) in the colon. Notably, butyrate, known to promote epithelial TGF-β production, was significantly elevated in colon and stool. Stool 16S rRNA gene sequencing revealed an enrichment of Lactococcus and Bacillus, genera that support butyrate production. These microbial shifts were accompanied by increased TGF-β abundance in colonic

epithelial cells, increased IL-10 in $CD4^+$ T cells and $Ly6C^+$ monocytes, and reduced $CD3^+$ T cell infiltration in the colon, suggesting attenuated alloreactivity. Beyond the gut, Modulen® IBD treatment enhanced IL-10 production in peripheral $CD4^+$ T cells and reduced $CD8^+$ T cell infiltration in the brain, indicating systemic immune-regulatory effects.

Conclusion Based on the above changes, we hypothesise that enrichment of Lactococcus and Bacillus contributes to increased colonic butyrate levels. This may support epithelial and innate immune-derived TGF-β production and IL-10 induction in $CD4^+$ T cells. While our data demonstrate strong associations, further studies (for e.g. with gnotobiotic models) are needed to establish definitive causal links. Our findings support Modulen® IBD as a promising non-pharmacological adjunctive therapy, providing a rationale for integrating it into clinical GI-aGVHD management.

P09.03 ROLE OF SYK IN REPROGRAMMING THE LME AND ENHANCING THE SUSCEPTIBILITY TO CAR-T CELL THERAPY

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10.1136/JITC-2026-ITOC.72

Background Chimeric antigen receptor (CAR) T cell therapy has emerged as a revolutionary therapeutic strategy in relapsed/refractory (r/r) aggressive B-cell non-Hodgkin lymphoma (B-NHL). However, around 60% of patients experience primary resistance or eventual relapse, highlighting a critical unmet clinical need. Multiple mechanisms contribute to CAR-T cell therapy failure, including CAR-T cell dysfunction, antigen escape and the immunosuppressive lymphoma microenvironment (LME), which is increasingly recognized as a key mediator of CAR-T cell therapy resistance. Previously, we identified lymphoma associated myeloid monocytic (LAMM) cells in the lymphoma microenvironment, which drive resistance against CAR-T cell therapy and are associated with worse clinical outcome. We found that LAMM cells show a high expression of spleen tyrosine kinase (SYK), indicating a relevant role of SYK signalling for LAMM cell function.

Methods We characterized LAMM cells through high-dimensional profiling combining single-cell RNA sequencing, bulk RNA sequencing and proteomics. To investigate the mechanism by which SYK inhibition (SYKi) modulates LAMM cell–mediated CAR-T cell inhibition, we conduct co-culture assays of LAMM cells under SYKi with anti-CD19 CAR-T cells. Additionally, we perform proteomic analysis to investigate the impact of SYKi on LAMM cells. The effect of combining SYK inhibition with anti-CD19 CAR-T therapy is assessed in an autochthonous DLBCL mouse model. We elucidate the underlying mechanisms by analysing collected tissue samples using a multiomic approach.

Results High SYK expression in LAMM cells is associated with shorter progression-free survival and non-durable responses. First in vitro data shows that SYKi hinders the proliferation and phenotype differentiation of LAMM cells. Our preliminary in vivo data demonstrates that combining SYK inhibition with CAR-T cell therapy prolongs survival in the DLBCL mouse model.

Conclusion Taken together SYK inhibition might be a promising strategy to enhance to CAR-T cell therapy and improve patient outcomes.

Dietz L : none, Orschel C: none, Kallinowski J: none, Prinz L: none, Stahl D: none, Schlözer L: none, Nill M: none, Mirjam K: none, Gholamipoorfard R: none, Flümann R : none, Riet T : none, Lindemeyer J : none, Grüll H : none, Büttner R: none, Borchmann P: none, Markus C: none, Reinhardt C: none, Abedpour N: none, Ullrich R: none

P09.04 ABLATION OF PROSTAGLANDIN E2-SIGNALING THROUGH DUAL RECEPTOR KNOCK-OUT IN CAR T CELLS ENHANCES THERAPEUTIC EFFICACY IN SOLID TUMORS

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10.1136/JITC-2026-ITOC.73

Background Chimeric antigen receptor (CAR) T cell therapy has revolutionized treatment for hematologic malignancies but remains largely ineffective against solid tumors due to the immunosuppressive tumor microenvironment (TME). Prostaglandin E2 (PGE2) is a central mediator of tumor-driven immune suppression, acting as a local inhibitor of T cell function. We hypothesized that targeted ablation of PGE2 signaling could enhance CAR T cell activity in PGE2-rich TMEs by rendering them insensitive to PGE2-mediated inhibition. To

test this, we disrupted PGE2 sensing via genetic deletion of its receptors EP2 and EP4.

Material and Methods Using CRISPR/Cas9 gene-editing, we generated CAR T cells double deficient for EP2 and EP4 (EP2-/-EP4-/-). Next generation sequencing (NGS) of the CRISPR loci was performed to assess on-target editing efficiency as well as whole genome sequencing (WGS) of a representative donor to assess potential off-target editing. PGE2-signaling downstream of EP2 and EP4 (cAMP production and CREB phosphorylation), proliferation, activation, and cytotoxic capacity in the presence or absence of PGE2 were assessed in vitro. In vivo efficacy was evaluated in both syngeneic and human xenograft tumor models, including patient-derived xenografts from pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and neuroendocrine tumors (NET). T cell intratumoral accumulation and persistence were monitored by flow cytometric analyses.

Results Successful generation of PGE2-insensitive EP2-/-EP4-/-CAR T cells was confirmed by NGS and showed complete abrogation of cAMP signaling and CREB phosphorylation upon PGE2 stimulation. No off-targets could be identified by WGS. While wild-type CAR T cells displayed impaired proliferation and reduced accumulation under PGE2 exposure, EP2-/-EP4-/-CAR T cells maintained robust proliferation and cytotoxicity. In vivo, loss of EP2 and EP4 markedly improved CAR T cell persistence and tumor infiltration, resulting in enhanced tumor control and prolonged survival across syngeneic and xenograft models. Notably, augmented anti-tumor activity was also observed when cocultured with patient-derived PDAC, CRC, and NET samples.

Discussion These findings identify PGE2 as a potent barrier to CAR T cell efficacy in solid tumors and demonstrate that dual genetic disruption of EP2 and EP4 effectively shields therapeutic T cells from PGE2-mediated immunosuppression. Single receptor knockouts were insufficient to achieve this effect, underscoring the cooperative nature of EP2/EP4 signaling. Unlike systemic pharmacologic inhibition of PGE2 pathways, CAR T cell–intrinsic receptor ablation avoids perturbing physiological PGE2 functions. Targeting PGE2 signaling thus represents a compelling gene-editing strategy to enhance CAR T cell resilience and broaden their applicability in solid cancer therapy.

S.K. has received honoraria from Plectonic, TCR2 Inc., Regeneron, Miltenyi, Galapagos, Cymab, Novartis, BMS and GSK. S.K. is an inventor of several patents in the field of immuno-oncology. S.K. and S.E. received license fees from TCR2 Inc and Carina Biotech. S.K. received research support from TCR2 Inc., Tabby Therapeutics, Catalym GmbH, Plectonic GmbH and Arcus Bioscience for work unrelated to the manuscript. R.W.J. is a member of the advisory board for and has a financial interest in Xsphera Biosciences Inc. R.W.J. has received honoraria from Incyte (invited speaker), G1 Therapeutics (advisory board), Bioxcel Therapeutics (invited speaker). R.W.J. has an ownership interest in U.S. patents US20200399573A9 and US20210363595A1. R.W.J.'s interests were reviewed and are managed by Massachusetts General Hospital and Mass General Brigham in accordance with their conflict-of-interest policies. The remaining authors declare no competing interests.

P09.05 ABSTRACT WITHDRAWN

P09.06 METABOLOMIC-DERIVED BIOMARKERS PREDICT IMMUNOTHERAPY EFFICACY IN ADVANCED NON-SMALL CELL LUNG CANCER

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10.1136/JITC-2026-ITOC.74

Background While immunotherapy improves survival in advanced Non-small cell lung cancer (NSCLC), predictive biomarkers are still needed. Emerging evidence suggests gut microbiota-related metabolites may influence treatment response, but their role in NSCLC remains unclear.

Methods This prospective study enrolled previously untreated wild-type NSCLC patients scheduled to receive immune checkpoint inhibitors. Clinicopathological features were recorded. Baseline fecal and blood samples were collected. Fecal and serum metabolites were analyzed through untargeted metabolomics.

Results 29 wild-type NSCLC patients were enrolled in the study. 808 differential metabolites between the two groups, of which 62 differential metabolites could be annotated. Responder enriched in L-Urobilin, Pentadecanoic acid, among others, while the non-responder enriched in L-Leucine, Glyceraldehyde, among others. Higher L-Urobilin levels were significantly associated with improved immunotherapy outcomes (P=0.0031) . Multivariate analysis established L-Urobilin as an independent prognostic factor (HR=0.88, 95% CI 0.78-0.99, p=0.0389). Plasma metabolomics further validated the predominant accumulation of L-Urobilin in the responder cohort.

Conclusion L-Urobilin may serve as a novel predictive biomarker for immunotherapy response in NSCLC. This study identifies a potential target for metabolomics-guided personalized immunotherapy.

Qing Zhou Received honoraria from: AstraZeneca, Lilly, Roche, Pfizer, Boehringer Ingelheim, MSD Oncology, Bristol Myers Squibb, Hengrui, BeOne Medicines Ltd, outside the submitted work. Other authors declare no competing interests.

P09.07 DUAL PTPN2/PTPN1 INHIBITION AUGMENTS BISPECIFIC T CELL ENGAGER ACTIVITY IN PATIENT-DERIVED TUMOR MODELS

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10.1136/JITC-2026-ITOC.75

Background Despite significant advances in cancer immunotherapy, a large unmet clinical need remains, as only a subset of patients achieves durable responses to existing treatments. To address this gap, novel targets are continuously being explored, including the protein tyrosine phosphatases PTPN2 and PTPN1. These critical intracellular immune checkpoints negatively regulate T cell receptor signaling and cytokine responses, thereby limiting antitumor immunity. The recent development of dual PTPN2/PTPN1 active-site inhibitors, such as ABBV-CLS-484 (AC484), has introduced a new strategy, demonstrating promising results in preclinical cell-based assays and mouse tumor

models.[1] This study investigates a novel combination of dual PTPN2/PTPN1 inhibition with a bispecific T cell engager to assess T cell activation and antitumor efficacy in patient-derived tumor model systems.

Material and Methods Two patient-derived model systems were employed to assess the combinatorial effects of AC484 and an EpCAM/CD3 bispecific T cell engager tool compound (EpCAM/CD3). Precision-cut tumor slices (PCTS) were generated from freshly resected colorectal cancer tissue and treated ex vivo for 48 hours. In parallel, malignant pleural and ascitic effusions containing tumor cells, fibroblasts, and immune cells were collected and cryopreserved. Tumor cell lines established from these effusions were subsequently co-cultured with autologous effusion-derived cells in the presence of AC484 and EpCAM/CD3 for 72 hours. Immune activation and tumor cell cytotoxicity were quantified by flow cytometry, while PCTS were further analyzed by immunohistochemistry and multiplex cytokine profiling of culture supernatants.

Results T cell activation (CD69) and reduced tumor proliferation (Ki67) induced by EpCAM/CD3 were further enhanced when combined with AC484 in PCTS. In malignant effusion co-cultures, single-agent treatment induced moderate tumor cell death, whereas combined treatment with EpCAM/CD3 and AC484 resulted in greater cytotoxicity. Consistent with these observations, multiplex cytokine profiling indicated a potentiated immune response in patient-derived models treated with the combination regimen.

Conclusion Our findings demonstrate that the novel combination of dual PTPN2/PTPN1 inhibition with EpCAM/CD3 significantly enhances T cell activation and tumor cell killing in patient-derived PCTS and malignant effusion co-cultures. These results support further investigation of this combination as a promising approach to enhance antitumor immune responses.

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P09.08 THE CD8+ T-CELL RESPONSE TRIGGERED BY NECTIN-4 AND CD137 LINKED BICYCLIC PEPTIDES

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10.1136/JITC-2026-ITOC.76

Introduction Compared to other immune checkpoint blockade therapies, co-stimulatory receptor agonising therapeutics for solid tumours have generated relatively underwhelming clinical trial results.

Some failures have been due to off-site effects resulting in unwanted stimulation of a host immune response. This has prompted development of bispecific T-cell engagers that confer specificity to a T-cell co-stimulatory receptor and a target of interest, often a tumour associated antigen (TAA), to provide tumour specific activation. CD137 (TNFRSF9/4-1BB) is a tumour necrosis factor receptor superfamily (TNFRSF) member with TCR activation-dependant expression that provides co-stimulation when bound by its native ligand, CD137L (TNFSF9/4-1BBL). Signalling through CD137 has commonly been characterised on CD8+ T-cells, with the TRAF binding region of CD137 now the preferred signalling domain on chimeric antigen receptor (CAR)-T-cells, improving cell longevity, proliferation and cytotoxicity when compared to CD28. Despite a breadth of research aimed at agonising CD137 on endogenous T-cells to enhance a host anti-tumour response, clinical trials with monoclonal antibodies have shown either lack of potency or severe toxicity.

Bicyclic peptide (Bicycle®) molecules are a novel therapeutic modality consisting of constrained bicyclic peptides of 1.5-2.5kDa in molecular weight with high affinity and specificity for a target antigen. Bicycle molecules can be connected by a flexible linker to enable bispecificity or toxic cargo loading. Bispecific tumour targeted immune cell agonists (Bicycle TICAs) are fully synthetic immunomodulators targeting both a TAA and a co-stimulatory receptor for site-specific cytotoxicity. In this study, we have characterised the effects of a novel CD137 and Nectin-4 specific Bicycle TICA® on human primary CD8+ T-cells.

Materials and Methods To mimic tumour antigen-mediated T cell activation, we have optimised a culture system utilising Fc receptor (FcRs) presented on target cells to bind the Fc region of anti-CD3, which stimulates the CD3 T-cell receptor (TCR) subunit on co-cultured CD8+ T-cells. To analyse the effects of a TICATM on T-cells undergoing TCR stimulation, we compare cultures with and without the TICATM using phenotypic assays, including ELISA, immunofluorescence and flow cytometry, as well as metabolic analyses, proliferation tracking, and RNA-sequencing.

Results We demonstrate that the Bicycle TICA® induces an altered state of co-stimulatory signalling, including CD137 self-feedback at both the protein and transcript levels. In addition, we find evidence for metabolic changes relating to enhanced glycolytic activity, mitochondrial size and action potential and bolstered rates for Nicatinomide adenoside dinucleotide (NAD) production that, together with other data, support enhanced CD8+ T-cell proliferative capacity.

Conclusion We find that a CD137 and Nectin-4 specific Bicycle TICA® molecule profoundly affects the effector CD8+ T cell response upon TCR challenge through altering the co-stimulatory landscape and increasing cellular proliferation. We also demonstrate enhanced expression and activity of metabolic machinery commensurate with robust signalling through CD137.

G. Smith: Babraham Institute, Bicycle Therapeutics: modest, M. Bell: Bicycle Therapeutics: significant; W. Lu: Bicycle Therapeutics: significant; I. Rioja: Bicycle Therapeutics: significant; A. Richard: Babraham Institute: None

P09.09 ADAPTER P329G-DIRECTED CAR T CELLS ENABLE EFFICIENT TARGETING OF LUNG CANCER CELLS IN LIVING HUMAN LUNG SLICES

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10.1136/JITC-2026-ITOC.77

Background Chimeric antigen receptor (CAR) T cell therapy has shown limited efficacy in solid tumors such as lung cancer due to antigen heterogeneity, antigen loss, immunosuppressive tumor microenvironments (TME), and on-target/off-tumor toxicities. Adapter CAR T cell platforms address these challenges by decoupling antigen recognition from CAR specificity. Anti-P329G CAR T cells recognize the P329G mutation in the Fc region of human IgG1 antibodies and can be flexibly redirected using P329G-Fc–mutated antibodies against different tumor antigens. Here, we evaluate the efficacy of anti-P329G adapter CAR T cells in lung cancer, with a focus on patient-derived ex vivo models that preserve tissue architecture and TME complexity.

Methods Second generation anti-P329G CAR T cells were combined with P329G-Fc–mutated antibodies targeting EGFR based on cetuximab and GA201. Conventional CAR and untransduced T cells were used as controls. CAR T cell effector functions were assessed in vitro and in vivo after stimulation with recombinant proteins or against antigen-expressing lung cancer cell lines. For ex vivo validation, two living human lung slices models were employed. Tumor-cell–seeded precision-cut lung slices (TCS-PCLS) were generated by introducing tumor and immune cells into healthy lung slices. Tumor-derived PCLS (TD-PCLS) were used as an ex vivo platform for studying the lung TME. Immunofluorescence staining and quantification of EdU as proliferation marker and Tunel as apoptosis marker were performed in the TCS-PCLS and TD-PCLS model after co-culture with anti-P329G CAR T cells.

Results Anti-P329G adapter CAR T cells mediated antigen-specific activation and cytotoxicity in vitro when combined with EGFR-directed P329G-Fc-mutated antibodies. The platform operated in a modular, antigen-specific and reversible manner. In lung cancer xenograft models, EGFR-redirected anti-P329G CAR T cells induced tumor remissions comparable to conventional anti-EGFR CAR T cells. The TD-PCLS model preserves the heterogeneity and metabolic activity of primary tumors, shows patient-specific responses and was used as an ex vivo platform for studying the efficacy of anti-P329G CAR T cells in a lung TME. Co-culture of anti-P329G CAR T cells with EGFR-targeting P329G-Fc-mutated antibodies in TCS-PCLS and TD-PCLS led to an efficient reduction of EdU+ tumor cells and increase of Tunel+ tumor cells compared to anti-P329G CAR T cells alone or the antibody alone. Therefore, anti-P329G CAR T cells can efficiently decrease proliferation and increase apoptosis of tumor cells in both PCLS models. In TCS-PCLS, no relevant toxicity against the healthy lung tissue part was seen, despite targeting a shared antigen, indicative of discriminatory potential of the platform.

Conclusion Anti-P329G adapter CAR T cells represent a versatile and effective approach for lung cancer immunotherapy. The successful application of the anti-P329G CAR platform in different PCLS models highlights its translational relevance and demonstrates the value of human lung slice models for evaluating CAR T cell therapies in complex, patient-relevant microenvironments.

M. Surowka: Previous employment and patents (significant): Roche. M. Subklewe: Research Grant (significant): Amgen, BMS/Celgene, Gilead/Kite, Janssen, Miltenyi Biotec, Novartis, Roche, Seattle Genetics, Takeda. Speakers Bureau/Honoraria (significant): Amgen, BMS/Celgene, Gilead/Kite, Novartis. Consultant/Advisory Board (significant): Janssen, Takeda, AstraZeneca, Pfizer, Ichnos Sciences, AvenCell, Incyte. M. von Bergwelt Baildon: Consultancy, Research Funding and Honoraria (significant): MSD Sharp & Dohme, Novartis, Roche, Kite/Gilead, Bristol Myers Squibb, Astellas, Mologen, and Miltenyi. C. Klein: Previous employment, stock ownership, patents (significant): Roche. S. Kobold: Research Grant (significant): TCR2 Inc., Arcus Biosciences, Tabby Therapeutics, Plectonic, Catalym GmbH. Speakers Bureau/Honoraria (significant): BMS, GSK, Novartis, TCR2 Inc., Miltenyi Biotech. Ownership interest/patents (significant): LMU University Hospital. Other (Significant): Carina Biotech, TCR2 Inc.. The remaining authors have nothing relevant for this submission to declare.

P09.10 COMPETITIVE MODULATION OF ANTI-P329G ADAPTER CAR T CELL ACTIVITY USING AN UNTARGETED CAR-ADAPTER MOLECULE

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10.1136/JITC-2026-ITOC.78

Background Chimeric antigen receptor (CAR) T cell therapy has demonstrated limited efficacy in treating solid tumors like lung cancer. This insufficient therapeutic response is mainly attributed to challenges such as antigen loss, antigen heterogeneity, and treatment-associated toxicities. The anti-P329G CAR T cell adapter platform combined with P329G-Fc-mutated antibodies provides a modular and flexible strategy to overcome these obstacles. Toxicity control can be achieved by interruption of the administration of the CAR-adapter molecule or by step-up dosing. Although antibody dosing enables partial control over therapy-associated toxicity, the capacity to modulate CAR T cell activity becomes limited once the antibody is present in the system. In this project, we aim to explore whether the

activity of anti-P329G adapter CAR T cells can be attenuated through the competitive administration of a non–antigen-binding, inert P329G-mutated CAR-adapter molecule.

Methods Second generation anti-P329G CAR T cells were activated with an EGFR-recognizing P329G-Fc-mutated antibody to evaluate the impact of varying concentrations and administration time points of a non–antigen-binding DP47-based antibody. Controls included CAR T cells exposed to a single antibody, unstimulated CAR T cells, and untransduced cells. In vitro activation following stimulation with recombinant protein or co-culture with EGFR-expressing lung cancer cells was assessed by measuring cytokine production using ELISA or FACS-based assays. Cytotoxic efficacy against tumor cells was measured with impedance-based cytotoxicity assays.

Results Anti-P329G adapter CAR T cells combined with the EGFR-targeting antibody demonstrated antigen-specific activation, cytokine release, and cytotoxic activity in vitro. Additional administration of the untargeted antibody led to a trend toward reduced cytokine production after stimulation with recombinant protein or co-culture with EGFR-expressing lung cancer cells. Despite this reduction, CAR T cells maintained full cytotoxic capacity in the presence of the untargeted antibody. Stepwise increase of the non–antigen-binding to antigen-binding antibody up to 1000x did not fully inhibit anti-P329G CAR T cell activity. Altering the sequence of antibody administration did not further enhance the reduction of activation. Whereas removal of the anti-EGFR antibody led to a relevant reduction of CAR T cell activation. Compared to discontinuing the administration of the EGFR antibody, the untargeted antibody did not provide additional benefit.

Conclusion Administration of an untargeted P329G-Fc-mutated antibody resulted in a slight trend toward reduced activation of anti-P329G adapter CAR T cells. However, this intervention did not provide additional benefit beyond that achieved by simple withdrawal of the anti-EGFR antibody administration. These findings indicate that step-up dosing and scheduled discontinuation remain the most effective strategies for toxicity control in adapter CAR T cell therapy. This approach further underscores the inherent advantages of adapter CAR T cells, particularly their modularity and potential for improved safety in immunotherapy.

M. Surowka: Previous employment and patents (significant): Roche M. Subklewe: Research Grant (significant): Amgen, BMS/Celgene, Gilead/Kite, Janssen, Miltenyi Biotec, Novartis, Roche, Seattle Genetics, Takeda. Speakers Bureau/Honoraria (significant): Amgen, BMS/Celgene, Gilead/Kite, Novartis. Consultant/Advisory Board (significant): Janssen, Takeda, AstraZeneca, Pfizer, Ichnos Sciences, AvenCell, Incyte. M. von Bergwelt Baildon: Consultancy, Research Funding, and Honoraria (significant): MSD Sharp & Dohme, Novartis, Roche, Kite/Gilead, Bristol Myers Squibb, Astellas, Mologen, and Miltenyi C. Klein: Previous employment, stock ownership, patents (significant): Roche S. Kobold: Research Grant (significant): TCR2 Inc., Arcus Biosciences, Tabby Therapeutics, Plectonic, Catalym GmbH. Speakers Bureau/Honoraria (significant): BMS, GSK, Novartis, TCR2 Inc., Miltenyi Biotech. Ownership Interest/patents (significant): LMU University Hospital. Other (Significant): Carina Biotech, TCR2 Inc. The remaining authors have nothing relevant for this submission to declare.

P09.11 LAMC2 DRIVES OSCC PROGRESSION BY REGULATING ERK/AKT-MEDIATED CELL CYCLE PROGRAMS AND PHENOTYPIC PLASTICITY

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10.1136/JITC-2026-ITOC.79

Background Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, with approximately 377,000 cases occurring worldwide each year.[1] Despite advances in treatment, the five-year survival rate remains as low as 20–30% for patients diagnosed at advanced stages. While the extracellular matrix (ECM) component Laminin subunit gamma-2 (LAMC2) is a known marker of poor prognosis, its role in coordinating the signaling programs that drive tumor aggressiveness and microenvironmental adaptation remains to be fully elucidated.[2] Understanding these ECM-driven mechanisms is essential for identifying novel translational targets to enhance the efficacy of immuno-oncology interventions in OSCC.

Material and Methods We integrated bioinformatic analysis of multiple OSCC datasets (GSE30784, GSE13601, GSE37991) to identify central hub genes. The functional impact of LAMC2 was validated in HSC-2 and HSC-4 cell lines and clinical specimens using qPCR, western blotting, and immunohistochemistry. Following siRNA-mediated knockdown, we performed proliferation and migration assays and analyzed ERK and Akt signaling dependencies. To investigate the biological function of LAMC2 in OSCC cells, we conducted RNA sequencing (RNA-seq) to identify transcriptomic shifts in immune-related pathways and epithelial–mesenchymal transition (EMT).

Results LAMC2 was identified as a critical hub gene significantly upregulated in OSCC tissue compared to dysplasia and normal oral epithelium. LAMC2 knockdown markedly inhibited cell proliferation and migratory capacity while reversing EMT phenotypes (reduced Vimentin/SNAI2, increased E-cadherin) in both cell lines. Immunohistochemical (IHC) analysis of 130 OSCC patient samples revealed a significant positive correlation between LAMC2 and Vimentin expression, suggesting a link between LAMC2 and the mesenchymal phenotype. Mechanistically, LAMC2 depletion suppressed the oncogenic p-ERK and p-Akt signaling axes. Notably, RNA-seq analysis revealed that LAMC2 silencing induced a major shift in the transcriptional landscape, upregulating the complement cascade while suppressing cell-cycle and DNA repair pathways. This suggests that LAMC2 expression may contribute to an evasive phenotype by modulating innate immune signaling pathways within the tumor stroma.

Conclusions These findings characterize LAMC2 as a central ECM-derived regulator that integrates intracellular signaling with broad transcriptional programs governing OSCC plasticity. By driving ERK/Akt-dependent EMT and modulating pathways related to the complement system, LAMC2 serves as a key architect of the pro-tumorigenic microenvironment. Targeting LAMC2-mediated signaling offers a promising translational strategy to remodel the ECM, overcome tumor-intrinsic resistance, and potentially sensitize OSCC to current immunotherapeutic approaches.

Funding This work was supported by JSPS KAKENHI Grants to T.K. (2023–2026) (JP23H03102)

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P09.12 CIRCULATING INFLAMMATORY MARKERS IN ENDOMETRIAL CANCER IN SURVIVAL PREDICITION

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10.1136/JITC-2026-ITOC.80

Background Endometrial cancer (EC) is the second most incident gynecologic cancer worldwide. Obesity is one of the most significant modifiable risk factors FOR EC, as it promotes chronic estrogen exposure and systemic inflammation, both of which contribute to endometrial carcinogenesis.

Several complete blood cell (CBC) derived indexes (CII) have been studied in different scenarios, receiving higher attention in the last years in oncology. Neutrophils (N), platelets (P), monocytes (M), and lymphocytes (Ly) each influence cancer by balancing inflammation and immunity—neutrophils/platelets often promote tumor-related inflammation, while lymphocytes provide anti-tumor defense and monocytes can become tumor-supporting macrophages. CII are easy tools to measure and visualize this imbalance in inflammatory cells.

Netosis is a process in which neutrophils release web-like structures of DNA and proteins that induce inflammation and create a pro-tumorigenic environment. cell-free DNA (cfDNA), therefore, functions as a surrogate marker of netosis.

The revised FIGO 2023 staging system incorporates anatomical extent, histopathological features, and molecular classification, offering a more precise framework.

Material and Methods 137 patients with EC were included at diagnosis, from 2018-2024. Data from routine CBC were retrieved from medical records. CII were calculated and investigated for outcome assessment. cfDNA was isolated from 5 mL of plasma with the QIAamp DNA Circulating Nucleic Acid Kit (Qiagen, Venlo, Netherlands) and quantified using Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA USA).

Statistical analysis was conducted using R Studio Version 2024.12.1 as well as IBM SPSS Statistics 31.0.0.0. Figures were generated using the R package ggplot2.

Optimal cut-off values were determined using the R packages survival and survminer, specifically with the surv_cutpoint function. Variables were subsequently dichotomized according to these cut-offs.

Univariate and multivariate Cox regression analyses were performed to evaluate the association of each variable with disease-free survival (DFS) and overall survival (OS).

Results In our cohort, cfDNA, L count, Ly count, ISS, PLR, MLR and PDW were statistically significant in the Kaplan-Meier and Cox analysis; whereas P count, N count, M count, RDW, HDW, AISI and SIRI did not achieve significant values.

The most complex indexes: AISI and SIRI, which include monocyte count did not prove statistically significant for DFS, despite being thoroughly studied and valuable in other solid tumors.

Conclusions In the last years CII have been subject of great attention in survival prediction of several solid tumors. Considering the inflammatory nature of endometrial cancer, inflammation markers may be of great importance both in the survival estimation as well as in guiding immunotherapy establishment and monitoring. Our results provide evidence of the value of complex CII indexes involving M, L, N and P, as well as cfDNA in DFS and OS evaluation in patients with EC.

P09.13 ARID1A LOSS IN FOLLICULAR LYMPHOMA DRIVES IMMUNE EVASION THROUGH ALTERED T-CELL INTERACTION

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10.1136/JITC-2026-ITOC.81

Background ARID1A, a key subunit of the SWI/SNF chromatin-remodeling complex, is mutated in 15% of follicular lymphomas (FL). These mutations are typically heterozygous and disruptive, and accumulate in advanced stages. ARID1A mutations are associated with inferior treatment outcomes and increased risk of transformation to aggressive clinical course. Previously, we demonstrated that ARID1A mutations enable evasion from FAS-mediated selection resulting in resistance to bispecific T-cell-engaging antibodies and CAR-T-cell therapy.[1] We hypothesized that ARID1A loss induces broader resilience programs in FL, enhancing lymphoma cell survival and altering interactions within the tumor microenvironment.

Methods CRISPR/Cas9-mediated heterozygous and homozygous knockout of ARID1A was achieved in three lymphoma cell lines (OCI-Ly1, OCI-Ly8, DB). Whole cell and surface proteomes were analyzed by mass spectrometry. Human germinal center (GC) B cells were isolated from tonsils and immortalized by BCL2 and BCL6 overexpression to generate FL-like cells (FLCs), still requiring YK6+CD40L+IL-21 feeder support for sustained growth. ARID1A knockdown was introduced by shRNA in FLCs using the RFP+pTRIPZ plasmid. FLCs were co-cultured with VPD-stained autologous T cells and analyzed by flow cytometry for conjugate formation, F-actin immunofluorescence stain for immune-synapse-formation and by RNA-sequencing of FACS-sorted FLCs and T cells and doublets.

Results Proteomic analysis of the lymphoma cell lines (Ly1, Ly8 and DB) demonstrated robust clustering by ARID1A genotype in the principal component analysis. A GO-term analysis of ARID1A-deficient cells revealed 'positive regulation of T-cell activation' (GO:0050870) as a top differentially regulated biological process in ARID1A-deficient cells. This signature included downregulation of LGALS9 (Galectin-9), a canonical HAVCR2/TIM-3 ligand implicated in modulation of T-cell expansion and effector function.

To model GC-derived FL biology and TME dependence we co-cultured the FLCs with autologous T-cells. ARID1A-deficient FLCs compared to wild-type cells formed significantly fewer conjugates with the T cells (Knockdown vs. Wildtype $p = 0.01$). In addition, an impaired cytoskeleton polymerization at the immune-synapse-formation was observed.

RNA sequencing of co-cultured T cells and FLCs showed that co-culturing had only minimal effects on the transcriptional programs of FLCs, but a profound effect on the transcriptomes of T cells. This suggests that ARID1A-dependent alterations in B cells primarily reshape T-cell responses.

Conclusion In our FL model systems, ARID1A loss alters the cell surfacome and reduces FLC-T cell conjugate and immune-synapse formation, accompanied by altered expression of T-cell regulatory surface proteins such as LGALS9. These changes likely modulate T-cell activation states and promote immune evasion. Ongoing multi-omics and functional studies aim to further dissect these mechanisms, and future work will correlate our findings with results from primary patient samples and explore therapeutic strategies to restore effective T-cell engagement in ARID1A-mutant FL.

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P09.14 PRELIMINARY IMMUNOPHENOTYPE ANALYSIS OF THE SYSTEMIC IMMUNE RESPONSE TO NEOADJUVANT IMMUNO-RADIOTHERAPY IN SOFT TISSUE SARCOMA

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10.1136/JITC-2026-ITOC.82

Background Soft tissue sarcomas (STS) are rare tumors of mesenchymal origin that usually do not respond well to immunotherapy due to their immunosuppressive tumor microenvironment ('cold tumors'). Therefore, an essential need for the development of other therapy approaches potentially effective in STS. The EFTISARC-NEO clinical trial (NCT06128863) explores the neoadjuvant combination of eftilagimod alfa (sLAG-3Ig, a potential APC activator), pembrolizumab (anti-PD-1 antibody, affecting the PD-1/PD-L1/2 axis) and radiotherapy. The rationale is to activate the immune response via eftilagimod alfa, while also 'unlocking' the immunosuppressive microenvironment via pembrolizumab. Additionally, the radiotherapy is supposed to increase the release of tumor neoantigens and therefore improve the antigen presentation and immune response.
Methods The preliminary results are available from 12 patients treated in the EFTISARC-NEO clinical trial (NCT06128863). The patients throughout the treatment received 3x200mg iv pembro, 5x30mg sc efti and 25x2Gy radiation. The peripheral blood mononuclear cells were isolated using the density gradient separation from samples collected before therapy (baseline) and 24h before surgery (post-treatment). The assessment of the PBMC immunophenotype was performed with two personalised multi-color panels of 18 fluorochrome-conjugated antibodies (BD Biosciences) and BD FACS Aria III flow cytometer (BD Biosciences).
Results We reported increased proliferative potential of both CD4+ helper and CD8+ cytotoxic T cells (increased percentage of Ki-67+ cells), including T cells with late activation markers (HLA-DR expression). Interestingly, after therapy we noticed increased percentage of CD4+ cells double-positive for GATA-3 and RORγt, indicating a potential intermediate state of Th2/Th17-like cells. The percentage of Treg cells (CD4+ FoxP3+) and early-activated Treg cells (CD4+ FoxP3+ CD25+) was significantly increased after therapy, which could be a sign of an induction of immune tolerance mechanisms. We did not report significant differences in the exhaustion profile (PD-1/PD-L1/CTLA-4/LAG-3 expression) in both CD8+ and CD4+ T cells. However, in the group of CD4+ effector memory T cells (TEM; CD45RO+, CD45RA-, CCR7-) we detected a decrease in the percentage of PD-1+ TEM after therapy, while the percentage of CTLA-4+ TEM increased, possibly as a sign of some compensatory exhaustion regulations.
Conclusions There are significant indications of activated systemic immune response; however, there are also signs of induced immunosuppressive mechanisms associated with the development of immune tolerance. Further research is planned to more precisely analyse the systemic immune response and its correlation with local response and therapy outcome.

Modest honoraria/consulting fees: Bristol-Myers Squibb (PS, KK, PR), Gilead (PS), Sandoz (PS), Novartis (PS, KK), Pierre-Fabre (PS, KK, PR), MSD (KK, PR), Sanofi (KK, PR), Philogen (PR), Pfizer (PR), Genesis (PR), Madison Pharma (PR), Merck (PR), AstraZeneca (PR), Orphan Europe (PR). Stock holding: Celon Pharma (PS). Other authors (AW, MS, ES) declare no conflict of interest.

P09.15 GENOMIC LANDSCAPE AND PREDICTIVE BIOMARKERS OF PRIMARY EGFR-TKI RESISTANCE AND LONG-TERM SURVIVAL IN ADVANCED EGFR-MUTANT NSCLC: IMPLICATIONS FOR IMMUNOTHERAPY STRATEGIES

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10.1136/JITC-2026-ITOC.83

Background The incidence of primary resistance to EGFR-TKI is 20 ~ 30%, but the mechanism of occurrence and the optimal therapeutic strategy after resistance are unclear. This population may have unique molecular or immune characteristics. The aim of this study was to investigate the genomic alterations, PD-L1 expression, and immunotherapeutic efficacy after EGFR-TKI resistance in patients with primary EGFR-TKI resistance.
Methods We retrospectively analyzed 98 patients with advanced EGFR-mutant NSCLC who developed primary resistance (disease progression) within 3 months after first- or second-line use of EGFR-TKI versus 137 patients who had disease progression within 18 months after use of EGFR-TKI. Baseline tumor samples were subjected to next-generation sequencing (520 gene panel) and PD-L1 immunohistochemistry (22C3 antibody). The efficacy of immunotherapy (objective response rate, progression-free survival, overall survival) after TKI treatment was evaluated.
Result Among the primary resistance cohort, 56 patients had comprehensive baseline NGS results, while 62 patients in the long-term survival cohort had complete NGS data. In the primary resistance group, concomitant genetic alterations included TP53 (68.4%), other EGFR variants (31.6%), MET (12.3%), RB1 (10.3%), and RBM10 (10.5%). The long-term survival cohort exhibited TP53 (53.3%), other EGFR variants (26.7%), LRP1B (15.0%), CTNNB1 (13.3%), and PIK3CA (10.0%). No statistically significant differences were observed in co-occurring genomic alterations between the two cohorts (all $P>0.05$). However, the primary resistance cohort exhibited a significantly higher number of total genomic alterations compared to the

long-term survival cohort (P=0.013). PD-L1 expression was assessed in 65 primary resistance patients and 103 long-term survivors, with significantly higher PD-L1 expression observed in the primary resistance cohort (P<0.001). Following EGFR-TKI progression, 22 patients from each cohort received immunotherapy. The primary resistance group showed superior progression-free survival (7.5 vs 3.9 months; HR: 2.955; 95% CI: 1.364-6.402; P=0.004) and a trend toward improved overall survival (29.4 vs 12.4 months; HR: 2.318; 95% CI: 0.925-5.813; P=0.066). The objective response rate was significantly higher in primary resistance patients (54.5% vs 13.6%; P=0.011).

Conclusion Our findings reveal unique genomic features associated with primary drug resistance in patients with EGFR mutations. The high expression of PD-L1 in primary drug-resistant tumors suggests mechanisms of immune evasion. These data support the rationale for the use of immunotherapy in a population of advanced EGFR-mutant lung cancers with primary resistance to EGFR-TKI. This suggests that the immune microenvironment may play an important role in primary resistance in patients with EGFR mutations.

Qing Zhou: Received honoraria from: AstraZeneca, Lilly, Roche, Pfizer, Boehringer Ingelheim, MSD Oncology, Bristol Myers Squibb, Hengrui, BeOne Medicines Ltd, outside the submitted work. Mingyi Yang: None.

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Die CAR-T-Zelltherapie mit Überlebensvorteil im RRMM[1,±]:

84,3%

30-Monats-OS-Rate[2,*,#]

Denken Sie frühzeitig an CARVYKTI®. Zur Übersicht zertifizierter Zentren: jjmc.link/a825zentren

***** Ergebnisse der multizentr., offenen, randomisierten Phase-III-Studie CARTITUDE-4 mit Lenalidomid-refraktären RRMM-Patient:innen nach 1–3 Vortherapien (CARVYKTI® (n = 208) vs. SOC definiert als PVd oder DPd (n = 211)), nach 33,6 Monaten medianem Follow-up. Prim. Endpunkt: PFS; sek. Endpunkte u. a.: ≥ CR, ORR, MRD-Negativität, OS. **#** Die 30-Monats-OS-Rate lag im CARVYKTI®-Arm bei 84,3% in der As Treated Population (n=176). Im gesamten CARVYKTI®-Arm betrug die OS-Rate 76,4% vs. 63,8% im SOC (HR 0,55; 95% KI,0.39–0.79; p = 0,0009). **±** CARVYKTI® ist indiziert für die Behandlung erwachsener Patient:innen mit rezidiviertem und refraktärem Multiplen Myelom, die zuvor bereits mindestens eine Therapie erhalten haben, darunter einen Immunmodulator und einen Proteasom-Inhibitor, und die während der letzten Therapie eine Krankheitsprogression zeigten und gegenüber Lenalidomid refraktär sind.

CAR: Chimärer Antigenrezeptor; **CR:** Komplettes Ansprechen; **DPd:** Daratumumab + Pomalidomid + Dexamethason; **HR:** Hazard Ratio; **KI:** Konfidenzintervall; **MRD:** Minimale Resterkrankung; **OS:** Gesamtüberleben; **ORR:** Gesamtansprechrate; **PFS:** Progressionsfreies Überleben; **PVd:** Pomalidomid + Bortezomib + Dexamethason; **RRMM:** Rezidiviertes/refraktäres Multiples Myelom; **SOC:** Standard of Care

1. Aktuelle Fachinformation CARVYKTI® **2.** Mateos MV et al., Overall Survival With Ciltacabtagene Autoleucel Versus Standard of Care in Lenalidomide-Refractory Multiple Myeloma: Phase 3 CARTITUDE-4 Study Update, Presented at the 21st International Myeloma Society (IMS) Annual Meeting; September 25-28, 2024; Rio de Janeiro, Brazil.

▼Dieses Arzneimittel unterliegt einer zusätzlichen Überwachung. Daher ist es wichtig, jeden Verdacht auf Nebenwirkungen in Verbindung mit diesem Arzneimittel zu melden.

CARVYKTI® 3,2 × 10^6 – 1 × 10^8 Zellen Infusionsdispersion. Wirkstoff: Ciltacabtagene autoleucel. **Zusammensetz.:** Infus.beutel enth. Ciltacabtagene autoleucel i. e. chargenabh. Konz. autol. T-Zellen. Sonst. Bestandt.: Cryostor CS5 (enth. Dimethylsulfoxid). **Anw.geb.:** Bhdlg. v. erwachs. Pat. m. rezidiv. u. refrakt. Multip. Myelom, d. zuvor bereits mind. eine Ther. erh. haben, darunter e. Immunmodulator u. e. Proteasom-Inhib., u. d. währ. d. letzt. Ther. e. Krankh.progress. zeigten u. ggü. Lenalidomid refrakt. sind. **Gegenanz.:** Überempfindl. gg. d. Wirkst. od. e. d. sonst. Bestandt. od. gg. Bestandt. i. Arzneim., d. i. Rahmen d. vorber. lymphozytendeplet. Chemother. u. d. unterstützenden Ther. eingesetzt werden. **Nebenwirk.:** bakt. Infekt., Infekt. d. ob. Atemw., Virusinfekt., Pneum., Neutropenie, Thrombozytopenie, Anämie, Leukopenie, Lymphopenie, Koagulopathie, Hypogammaglobulin., Zytokin-Freissetzungssyndr., Hypokalz., Hypophosphat., verm. Appetit, Hypokali., Hypoalbumin., Hyponatri., Hypomagnesi., Hyperferritin., Enzephalopathie, Immuneffektorzell-assoz. Neurotoxiz.syndr., motor. Funkt.störg., Schwindelgef., Kopfschm., Schlafst., Tachyk., Hypotonie, Hypertonie, Blutung, Hypoxie, Dyspnoe, Husten, Diarrhö, Übelk., Erb., Obstipat., Schm. d. Muskel- u. Skelettsystems, Fieber, Fatigue, Schüttelfrost, Ödem, Schm., Transaminasen u. Gamma-Glutamyltransferase i. Blut erhöh. Sepsis, Gastroenterit., Harnwegsinfekt., Pilzinfekt., sek. Malignom m. myeloid. Ursprung, febr. Neutropenie, Lymphozyt., hämophagozyt. Lymphohistiozyt., Delirium, Persönlichkeitsveränd., Aphasie, Hirnnervenlähmg., Parese, Ataxie, Tremor, Neurotoxiz., periph. Neuropath., Herzrhythmusstörg., Thromb., Kapillarlecksyndr., Abdominalschm., immunvermitt. Enterokolitis, Hyperbilirubin., Hautausschl., Nierenvers., C-reakt. Protein erhöh., alkal. Phosphatase i. Blut erhöh., Guillain-Barré-Syndr., sek. Malignom m. T-Zell-Ursprung. **Warnhinw.:** Nur z. autol. Anw. **Verschreibungspflichtig. Pharmazeut. Unternehmer:** Janssen-Cilag International NV, Turnhoutseweg 30, 2340 Beerse, Belgien. **Örtl. Vertreter für Deutschland:** Janssen-Cilag GmbH, Johnson & Johnson Platz 1, 41470 Neuss. **Stand d. Inform.:** 07/25.

Janssen-Cilag GmbH

CP-536678, Stand: 09/2025