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MEETING REPORT

CIMT 2021: report on the 18th Annual Meeting of the Association for Cancer Immunotherapy

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ABSTRACT

After one year of absence, the 18th Annual Meeting of the Association for Cancer Immunotherapy (CIMT), Europe's cancer immunotherapy meeting, took place virtually from 10 to 12 May 2021. Over 850 academic and clinical professionals from 30 countries met to discuss the recent advancements in cancer immunotherapy and the current progress in COVID19-related research. This meeting report summarizes the highlights of CIMT2021.

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Introduction

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Cellular therapy

Allogeneic CAR T cells derived from healthy donors harbor the potential to overcome current clinical and manufacturing limitations. However, for complete prevention of allogeneic CAR T cell rejection by the host immune system, 3–6 engineering steps are required including MHCI/MHCII depletion to abolish T cell recognition and introduction of HLA-E, HLA-G or Siglec ligands to prevent NK cell-mediated killing.[1] **Tobias Feuchtinger (University Hospital Munich LMU, Germany)** and his colleagues focused on another relevant challenge – the prevention of graft-vs-host alloreactivity by knockout of the endogenous TCR. They developed a protocol comprising retroviral transduction of pre-activated T cells with a CD19-specific 41BB CAR construct followed by CRISPR/Cas9-based TCR knockout.[2] Since even minimal residual TCR positive cells can cause severe GvHD,[3] they included a CD3 deletion step post-expansion of engineered T cells. *In vitro* the introduction of the TCR knockout does not affect CAR T cell functionality but completely abolish alloreactivity to mismatched PBMCs. However, in both a patient-derived xenograft ALL (PDX) and a Nalm6 xenograft *in vivo* model, the TCR negative CAR T cells are not able to persist and only show a transiently antitumor effect. In contrast, conventional CAR T cells show long-term tumor control but also induce fatal alloreactivity (GvHD). In conclusion, further studies on allogeneic CAR T cells are required to clarify the role of the endogenous TCR and ensure safety and long-term clinical responses.

To improve TCR therapy for B-cell malignancies, the team of **Mirjam Heemskerk (Leiden University Medical Center, Netherlands)** established a platform for identification of promising TCR candidate peptides derived from lineage or tumor-specific genes. They isolated peptide-HLA complexes from primary tumor material via affinity chromatography and eluted the peptides for identification of the related genes via mass spectrometry. For promising target candidates, peptide-HLA tetramers were generated and used for single cell sorting of peptide-specific $CD8^+$ T cells from target HLA-negative healthy donor PBMCs. Finally, they sequenced TCRs of expanded T cell clones. Using this pipeline, they identified a HLA-B7 restricted BOB1-specific TCR that upon retroviral transfer in human $CD8^+$ T cells shows high antitumor reactivity *in vitro* and in a xenograft multiple myeloma (MM) *in vivo* model.[4] In their library, they also found functional TCRs specific for the constant domains of immunoglobulins, which represent promising targets for MM treatment.[5] Furthermore, Mirjam Heemskerk presented NK TCR cells as a potent tool to overcome major challenges in TCR T cell therapy, such as TCR mispairing, tumor-escape via HLA downregulation and risk of cytokine release syndrome.[6] Their two-step retroviral

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transduction protocol for primary NK cells comprises introduction of first a TCRα-TCRβ-CD8α-CD8β and second a CD3ζ-CD3δ-CD3ε-CD3γ construct under repetitive stimulation via K526 cells, membrane-bound IL21 and 41BBL. The resulting CD8, CD3, and BOB1-TCR expressing NK cells show TCR-dependent killing of tumor cells *in vitro* even in case of b2m knockout-induced HLA downregulation.

Johanna Olweus (University of Oslo, Norway) and her colleagues have already previously shown that in an allogeneic situation, T cells do not recognize only the foreign HLA itself, but instead recognize the complex of HLA and peptide presented in the groove, as in conventional TCR-recognition.[7] Hence, by selecting allo-restricted TCRs recognizing foreign HLA presenting a self-peptide, autologous tolerance to tumor-specific self-peptides can be bypassed. Her group identified terminal deoxynucleotidyl transferase(TdT)-peptide specific TCRs that after transfer to $CD8^+$ T cells mediate tumor cell recognition only in case of peptide presentation via HLA-A*02 :01.[8] TdT can be considered as a promising target for acute lymphocytic leukemia since it is not expressed in normal naïve or memory T or B cells, or in hematopoietic stem cells, but overexpressed in 80–90% of all B-ALL and T-ALL cases. In a combinatorial approach using *in vitro* experiments and bioinformatics, the group did not detect cross-reactivity of the identified TdT-TCRs with related peptides of the human proteome. TdT-TCR-transduced $CD8^+$ T cells show great anti-leukemic responses in NALM-6, BV173, and primary B-ALL xenograft mouse models, and importantly do not induce toxicity on normal human hematopoiesis neither in huNSG mice nor in *in vitro* colony formation assays. Johanna Olweus suggested that the absence of toxicity toward T cell progenitor cells can be explained by the asynchronous expression patterns of TdT and HLA-A2 during thymocyte development. Taken together, she concluded that TdT-TCR T cell therapy could be applicable for patients relapsing with CD19-negative B ALL after CAR T therapy and patients relapsing with T-ALL following chemotherapy.

Neoantigens and cancer vaccines

While being a key player in stimulation of antitumoral immune responses, IFNγ produced by tumor-infiltrating T cells contributes to immunosuppression by inducing indoleamine 2,3-dioxygenase 1 (IDO1), which catabolizes tryptophan into kynurenine. In clinical trials, inhibition of IDO1 in combination with PD-1 blockade in patients with melanoma did not lead to any superior treatment efficacy compared to PD-1 blockade alone. **Yardena Samuels (Weizmann Institute of Science, Israel)** began her talk by sharing her findings on the role of IDO1 as well as consequences of IFNγ-induced tryptophan degradation.[9] They observed that prolonged exposure of melanoma cells to IFNγ caused IDO1-induced tryptophan depletion. Based on ribosome profiling, they showed that the ribosomes were stalled at the translation start site, paused on tryptophan codons, continued translation despite the low Tryptophan levels and then paused again ~15 amino acids later, that is, W-bumps. These were later characterized by ribosomal frameshifting events. These frameshifted peptides were not only detectable in full proteomes but also were shown to be presented on HLA-I molecules on melanoma cells and shown to be immunogenic. Altogether, Samuels's findings add a new layer of complexity to the peptidome landscape of melanoma cells through presentation of potentially immunogenic peptides, which are generated via IDO1-mediated tryptophan depletion. Next, Samuels focused on the identification of microbial-derived HLA-bound peptides in melanoma.[10] They used 16S ribosomal RNA sequencing to identify the tumor microbiome and performed HLA peptidomics for identification of microbial-derived peptides presented on HLA class I and class II molecules in melanoma tumors. They identified numerous such presented peptides and observed that the hydrophobicity of the peptide antigens derived from bacteria correlated with the immunogenicity. In the future, Samuels and her team will focus on how bacteria are affecting the pathogenesis and immunotherapy response.

Although personalized cancer vaccines against patient-specific neoantigens serves as promising tools for cancer treatment, one needs to find optimal formulation, platform (RNA or peptide-based), and delivery route, e.g. intravenous or subcutaneous, to induce a neoantigen-specific T cells response. **Robert Seder (NIH-National Institute of Allergy and Infectious Diseases, USA)** focused on strategies for an improved peptide vaccine against neoantigens to achieve sufficient magnitude and breadth for *in vivo* effects. First, he focused on how the solubility of a neoantigen has an influence on the immunogenicity. He used two cancer neoantigens, that is, Reps1 and Itgb, identified from the colorectal cancer cell line MC38, by Yadav and colleagues.[11] While Reps1 neoantigen was immunogenic and insoluble, Irgq was non-immunogenic and soluble. Once they replace the central core sequences of Reps1 and Irgq to reverse their solubility characteristics, they were also able to reverse their immunogenicity, for example,, soluble Reps1 became non-immunogenic. Based on these findings, his team developed a self-assembling nanoparticle vaccine (SNP-7/8)[12] that is composed of a peptide neoantigen flanked by cathepsin cleavage sites, a charged molecule to allow solubilization of the peptide in the solution and a hydrophobic peptide linked to TLR7/8. Seder shared his finding on how vaccination route influenced the phenotype of T cells, that is, effector like or stem like T cells. While subcutaneous delivery (SNP-SC) prolongs antigen presentation with terminally differentiated effector-like T cells (GZMB, Klrg1, CX3CR1), intravenous delivery (SNP-IV) provides a transient antigen presentation with stem-like T cell phenotype (TCF1, Tox, Xcl1).

To achieve a sustained T cell response leading to durable immunity is one of the major aims of successful neoantigen vaccination. **Patrick Ott (Dana-Farber Cancer Institute and Harvard Medical School, USA)** shared his follow-up analysis, 4 years post treatment, from the NeoVax trial (NCT01970358),[13] a personalized neoantigen vaccine monotherapy for patients with melanoma.[14] The vaccine-induced strong $CD4^+$ and weaker $CD8^+$ T cell responses in more than 60% of the immunizing peptides used, even after median 2 years following the treatment.[13] They showed that vaccination-induced T cell responses were highly specific for the mutated epitope and in some cases against autologous tumors. Ott's findings revealed that all eight patients were alive post

NeoVax and 6/8 do not show any evidence of the disease.[14] Ott analyzed single-cell transcriptomic signatures of tetramer-specific T cells to investigate further the functional states of circulating vaccine-induced neoantigen-specific T cells. Four transcriptionally defined clusters comprised of cells from all three patients were observed. Additionally, neoantigen vaccine-specific TCR clonotypes tested on a single-cell level diversified throughout vaccination. Personal neoantigen vaccines were also found to trigger epitope spreading.

COVID-19 – immuno-oncology cross-talk

Christian Drosten (Charité, Germany) opened the COVID-19 – Immuno-Oncology Cross-Talk session and summarized the current state of knowledge about severe acute respiratory syndrome corona virus (SARS-CoV)-2 variants of concern. First, he focused on the distribution of virus variants in Germany, where the B.1.1.7 lineage currently dominates and outcompetes all other SARS-CoV-2 variants. B.1.1.7 is described as a variant with increased transmissibility and associated with higher relative disease severity, if 28 days risk of death or hospitalization are taken into account.[15–18] The virological characteristics underlying the increased transmissibility of B.1.1.7 are under investigation. *In vitro* studies in traditional systems like Vero E6 cells, human cell lines and primary human airway epithelial cells show that B.1.1.7 does not outcompete other contemporaneous SARS CoV-2 strains in terms of replication.[19] On the contrary, patients infected with B.1.1.7 have higher viral loads than non-B.1.1.7 infected patients.[17] Longitudinal PCR tests performed in individuals infected with SARS-CoV-2 revealed that B.1.1.7 may cause longer infections compared to non-B.1.1.7 SARS CoV 2.[20] Accordingly, B.1.1.7 produced higher levels of infectious virus late in infection in human upper respiratory tract, alveolar, and intestinal organoid models. These studies suggest that extended duration of infection, resulting in higher viral load, may be responsible for increased transmissibility of B.1.1.7.[21] Since B.1.1.7 does not show widespread immune escape, Christian Drosten assumed that SARS-CoV 2 variants with relevant immune escape have a selection advantage, which becomes increasingly important. He highlighted latest sequencing data from the United Kingdom, where the majority of adults is vaccinated at least once. There, other virus variants started outcompeting B.1.1.7. Additionally, in B.1617 (India) and B.1351 (South Africa) SARS CoV-2 variants with presumably stronger immune escape properties than B.1.1.7 dominate the infectious landscape. Christian Drosten hypothesized that preexisting immunity caused by natural infection with SARS-CoV-2 may not be sufficient to prevent resurgence of the pandemic. He referred to seroprevalence and humoral immune durability of anti-SARS CoV-2 antibody data from Wuhan, China, and India, where immunity against SARS CoV-2 appears to by transient.[22,23] Consequently, he underlined the importance and efficacy of vaccination. Recent data show that different types of COVID-19 vaccines induce robust neutralizing antibody responses against various SARS CoV-2 variants of concern.[24,25] Cellular immunity induced by mRNA vaccines is not substantially affected by mutations found in the SARS-CoV 2 variants.[26]

In his talk Josef Penninger (University of British Columbia, Canada) focused on angiotensin-converting enzyme (Ace) 2 and its relation to SARS-CoV-2 infections and COVID 19. Ace2 is an ectoenzyme expressed on the cell surface of endothelial and other cell types. Ace2 counterbalances Ace and thus provides homeostatic regulation of angiotensin II. Ace2 was furthermore identified as essential entry receptor for SARS-CoV-1 and SARS-CoV-2 into the cell. Josef Penninger and his colleagues developed soluble recombinant human Ace2 (rhAce2) for reduction of SARS-CoV-2 infections. Binding of rhAce2 to the viral spike protein should prevent viral contact to endogenous Ace2 on the cell membrane and subsequent viral entry. In Vero E6 cells, rhAce2 reduced SARS-CoV-2 loads by factor 1,000–5,000. rhAce2 could inhibit direct SARS-CoV-2 infection of human kidney and engineered human blood vessel organoids expressing Ace2.[27] In a phase II trial with 180 participants, rhAce2 was well tolerated and showed tendency of efficacy (NCT04335136). In Vero E6 cells and human kidney organoids combination therapy of Remdesivir and rhAce2 had an additive effect and a broadening of the respective therapeutic windows was observed.[28] Josef Penninger highlighted that therapy targeting the viral spike protein may suffer from viral escape mechanisms. However, the virus is not able to escape Ace2 binding and possibly selects for variants with a stronger Ace2-binding affinity. Thus, rhAce2 may also be efficient against different SARS-CoV-2 variants. Conclusive unpublished data show that rhAce2 nearly completely inhibits infections of Vero E6 cells with B.1.1.7 and B.1351 SARS-CoV-2 variants.

Keynote lecture

In his keynote lecture, **Ugur Sahin (BioNTech SE, Germany)** presented a roundup from the discovery of the mRNA in 1961 to its successful worldwide application as a COVID-19 vaccine. In 1996, Eli Gilboa demonstrated the feasibility of tumor-derived mRNA-pulsed dendritic cells (DCs) to induce immune responses against tumors.[29] This work inspired Ugur Sahin to systematically investigate the uptake of mRNA by DCs. He found that upon intranodal delivery naked antigen-encoding mRNA is taken up by DCs via micropinocytosis and induces antigen-specific $CD4^+$ and $CD8^+$ T cells.[30] However, to facilitate the *in vivo* application, formulations were required to deliver the mRNA to lymphoid DCs upon intravenous injection. Based on rational design, Ugur Sahin and his team developed liposome formulated mRNA (RNA-LPX) with a specific size and charge, which guides the mRNA to DCs in secondary lymphoid organs.[31] Apart from formulations, early mRNA vectors were far not sufficient to induce significant immune responses. Systemic studies modifying the vector backbone improved stability and translation yielding a 5000-fold higher potency.[32,33] Modification of nucleosides and purification of double-stranded RNA enabled engineering of mRNA-mediated immune effects.[34,35] With all these tools at hand, Ugur Sahin presented a variety of examples describing the application of specifically designed mRNA for treatment of cancer, autoimmunity and infectious diseases. In preclinical tumor models, antigen-encoding RNA-LPX primed tumor-specific T cell responses and induces Interferon-α due to toll-

like receptor 7 triggering.[31] In patients, a combination of four melanoma-associated antigens encoding RNA-LPX resulted in objective responses alone or in combination with anti-PD-1 in patients, which failed prior anti-PD-1 treatment.[36] The RNA-LPX technology was further applied to overcome limitations in CAR T-cell therapy of solid tumors. In mice, Claudin-6 (CLDN-6)-specific CAR T cells could be repetitively expanded by CLDN-6 RNA-LPX treatment and achieved regression of large tumors at subtherapeutic CAR T-cell doses.[37] Ugur Sahin highlighted a third concept of RNA-based cancer therapy, which is based on individualized mutanome vaccines implementing a poly-neoepitope approach.[38] Data from clinical trials revealed induction of poly-epitopic $CD4^+$ and $CD8^+$ T-cell responses and patients receiving vaccination had a reduced rate of metastatic relapses. Currently, two clinical studies in multiple cancer indications are ongoing (NCT03815058; NCT03289962). Subsequently, Ugur Sahin demonstrated that the RNA-LPX technology is feasible for the induction of tolerance as an active mechanism. Application of 1-methylpseudouridine modified RNA-LPX enables expression of the encoded protein in DCs under non-inflammatory conditions.[39] In the murine model of experimental autoimmune encephalomyelitis (EAE), application of modified RNA-LPX expanded antigen-specific Tregs and induced bystander immune suppression, subsequently leading to reversal of EAE. In the last part of his talk, Ugur Sahin focused on the development of the BioNTech SE COVID-19 mRNA vaccine. With the SARS-CoV-2 outbreak in December 2019, Ugur Sahin was concerned and dedicated all available forces to the development of an effective and safe vaccine. Based on the knowledge that neutralizing antibodies can be generated against the spike protein of SARS-CoV-1, 20 different immunogen designs of the SARS-CoV-2 spike protein were tested. The favorable candidates were formulated as Lipid Nanoparticles (LNPs) for intramuscular injection. Clinical trials started in April (Germany) and May (USA) 2019. Spike protein-specific IgG responses, stable virus neutralizing antibody titers as well as $CD4^+$ and $CD8^+$ T cell responses, which are poly-specific against different regions of the spike protein, were observed.[40–43] Importantly, efficacy of the vaccine is mostly maintained against SARS-CoV-2 variants.[25] Ugur Sahin emphasized that versatility of the mRNA, competencies and expertise in the RNA field as well as close collaboration with partners and regulatory authorities enabled the fast development of a COVID-19 vaccine, which prevents infections and protects from severe disease.

CIMT immunoguiding program

Noel de Miranda (Leiden University Medical Center, The Netherlands) focused his talk on identification and characterization of T cells, which elucidate antigen responses in Colorectal cancer (CRC). He also summarized the use of multidimensional characterization of the tumor microenvironment (TME) to understand the cellular relations between immune cells and tumor cells and identify additional immune cell subsets in the TME with antitumor activity. Previous findings highlight clinical activity of anti–PD-1 therapy in CRC with mismatch repair deficiency, a subtype with a high tumor mutational burden, as compared with the CRC subtype with mismatch repair proficiency, which has a significantly lower tumor mutational burden and a poor response to these agents.[44,45] Neol's group focuses on finding immunotherapeutic options for CRC subtype with mismatch repair proficiency. Neoantigen-targeted reactivity by autologous T cells in mismatch repair-proficient colorectal cancers of the CMS4 subtype has been identified.[46] When comparing the transcriptional profile of CMS2/3 subtype with CMS4 subtype, transforming growth factor-β (TGF-β)-related genes were seen to be upregulated. Recently, Neol's team published a review concluding that potentially TGF-β targeting in combination therapies with immunotherapy is a potential option to treat certain special cancer types.[47] He highlighted that they have embarked on an approach of utilizing high-dimensional analysis to provide an unbiased characterization of the immune contexture of CRC. They observed tumor tissue-specific immune signatures across the adaptive and innate compartments and found a previously unappreciated innate immune cell population (γδ T cells and Innate Lymphoid cells) implicated in antitumor immunity that strongly differentiated immunogenic (MMR-deficient) from non-immunogenic (MMR-proficient) CRCs.[48]

The next speaker, **Ping-Chih Ho (Ludwig Institute for Cancer Research, Switzerland)**, spoke about the strategy of metabolic targeting of intratumoral Tregs that can support to reprogram the TME. Studies have shown that high Treg infiltration associates with poor prognosis and more suppressive TME. However, systemic Treg cell depletion might increase the risk of immune-related adverse events (irAE), such as autoimmunity-related toxicities.[49] Hence, Ho and group focused on selectively targeting and demolish of intratumoral Tregs. Upon analysis of Treg RNAseq data, an upregulation of lipid metabolism associated genes in intratumoral Tregs was seen. The team further investigated differentially expressed genes and concluded that CD36 was selectively upregulated in intratumoral Treg cells as a central metabolic modulator. In murine melanoma models, it was observed that depletion of Treg-specific CD36 leads to decreased lipid uptake of intratumoral Tregs and resulted in suppression of tumor growth. In line with these findings, CD36 knock out did not negatively affect the functional suppressive activity in colitis autoimmune mouse models. Upon studying the mechanism Ho and colleagues concluded that CD36 fine-tunes mitochondrial fitness via peroxisome proliferator-activated receptor-β signaling and reprograms Treg cells to adapt to a lactic acid-enriched TME. Genetic ablation of CD36 in Treg cells suppressed tumor growth accompanied by a decrease in intratumoral Treg cells and enhancement of antitumor activity in tumor-infiltrating lymphocytes without disrupting immune homeostasis. Furthermore, CD36 targeting elicited additive antitumor responses with anti-programmed cell death protein 1 therapy.[50]

Although immune checkpoint blockade (ICB) therapy showed significant clinical response rates, there are still a major portion of melanoma patients which do not respond to such therapies.[51] The final speaker of the session **Göran B. Jönsson (Lund University, Sweden)**, emphasized to focus on non-responding patient cohorts. In a study comprising 177 ICB naïve melanoma patients, they found beside $CD8^+$ T cell

infiltration (in 33% of patients) also tumor associated $CD20^+$ B cells (in 25% of patients). Interestingly, these $CD20^+$ B cells formed tight "aggregates" in the tumor and were always accompanied by $CD8^+$ T cell infiltration. In deeper analysis, these aggregates showed signatures of tertiary lymphoid structures (TLS).[52] It has already been shown that tumor-associated B cells can be organized in TLS, which occur in different maturity states namely early or immature, primary follicle like or secondary follicle like.[53] Göran and his colleagues hypothesized that TLS might boost antitumor response by induction of antibody-producing B cells. Survival data from the cohort showed that the presence of TLS correlated with highest survival rates, followed by patients with only $CD8^+$ T cell infiltration and lastly patients without $CD8^+$ T cells and TLS. Upon using spatial profiling, the team observed two major groups of B cell phenotypes, $Ki67^{high}$ and $Ki67^{low}$. They also noted that $Ki67^{high}PD1^{high}CD40^{high}$ $MCHII^{high}$ tumor-associated B cells might operate in germinal centers and belong to more mature TLSs. Jönsson's team studied marker profiles for various TLS stages and hence developed gene signatures reflecting melanomas with TLS. They applied this gene signature to clinical data and observed that TLS^{high} signature patients have the best survival.

Microbiota

Our intestines host millions of microbes that can be cumulatively called gut microbiota or more provocatively the "invisible organ." Gut microbiota has various functions for the regulation of immune responses not only in gut but also at the systemic level. Can we also exploit microbiota for augmenting the efficacy of cancer immunotherapies? One answer to this question was provided by **Laurence Zitvogel (Institute Gustave Roussy, France)** who investigated the predictive role of Akkermansia muciniphila (A. muciniphila) for response and resistance to PD1 checkpoint blockade in non-small cell lung cancer confirming a previously published study by the same group.[54] While the normal relative abundance of this bacterium in the microbiota predicted sensitivity to the therapy, the high relative abundance predicted resistance to therapy in prospective cohort of 466 patients. Moreover, using multivariate Cox logistic regression analysis, she could show that this biomarker overrules PD-L1 and antibiotics to predict clinical benefit to PD1 blockade. These findings were further supported by a phase 2 randomized trial in non-small cell lung cancer in which the presence of A. muciniphila was found to be associated with major pathologic response to PD1 and CTLA4 dual blockade and positively correlated with TCR clonality in patient tumor samples.[55]

The gut microbiome regulates clinical responses to immune checkpoint blockade[56,57] by regulating the tumor microenvironment. **Hassane Zarour (UPMC Hillman Cancer Center, USA)** showed that fecal microbiome transplant (FMT) can be used to improve our understanding of commensal bacteria contribution to therapy success. FMT combined with anti-PD1 therapy-induced clinical responses in PD-1 refractory melanoma patients modulating systemic and intratumoral immunity in responders.[58] Interestingly, responders exhibited a distinct circulating cytokine, chemokine, and metabolic signature including decreased IL8 and increased IL12 and CXCL13 as well as $CD8^+$ T cell activation. Dr. Zarour argued that PD-1 refractory patients who also did not benefit from FMT could lack the ability to mount an efficient immune response regardless of microbiota composition or FMT did not include the taxa needed for anti-PD1 therapy effectiveness as well as failed to successfully implant into the recipient to induce the perturbation of the existing host microbiota.

Following a similar path with the previous talk, **Fyza Shaikh (Johns Hopkins School of Medicine, USA)** presented novel bacterial signatures identified by reanalysis of data across all cohorts using a standardized bioinformatics methodology comparing responders and non-responders from previously published immune checkpoint therapy clinical studies.[59] Employing the existing 16s rRNA amplicon and metagenomics sequencing data, her team could identify novel bacterial biomarkers and interestingly the non-responder associated integrated index, which showed to be the most effective signal in their uniform computational model. This strategy offers the potential to identify patients who might not initially respond to checkpoint therapy and reallocate them in microbiome-based interventions to improve response rates.

Tumor microenvironment

Opening the tumor microenvironment session, **Sjoerd van der Burg (Oncode Institute and Leiden University Medical Center, Netherlands)** highlighted their studies on the TME in oropharyngeal squamous cell cancer (OPSCC). Van der Burg and colleagues previously connected the presence of Human papillomavirus 16 (HPV16) specific $CD4^+$ and $CD8^+$ T cells to beneficial prognosis for $HPV16^+$ OPSCC patients.[60] Now they used bulk and single-cell transcriptomics as well as Imaging Mass Cytometry to compare the TME of patients with (IR+) or without such an immune-response (IR-) and impressively demonstrated a link between the chemokine-driven, spatially organized tumor immune infiltrate and survival of patients with OPSCC.

The work of **Ana Anderson (Harvard Medical School, USA)** and her team, presented in this talk, focused on dysfunctional $TIM3^+$, $PD\text{-}1^+$, $CD8^+$ T cells. Initially observing increased expression of Nr3c1, a glucocorticoid receptor gene, in $TIM3^+$, $PD\text{-}1^+$, $CD8^+$ T cells, Anderson and her team furthermore found an association between glucocorticoid signaling and T-cell dysfunction signatures using single cell sequencing. Conditional knock out of glucocorticoid signaling in mature $CD8^+$ T cells ($NR3C1fl/flE8iCre^+$) led to improved tumor growth control, accompanied by a change in effector phenotype, which was characterized by increased pro-inflammatory cytokine secretion, increased cytotoxic capacity, and decreased checkpoint receptor expression. Despite steroids normally being produced centrally in the adrenal cortex, Anderson and colleagues found tumor-associated monocytes/macrophages producing glucosteroids in tumor tissue, as seen by the expression of enzymes for steroid synthesis. Genetic ablation of steroidogenesis in these cells was sufficient to induce a $CD8^+$ effector phenotype similar to the previously described KO model. Lastly, Anderson showed that a high glucocorticoid signature score could be seen in biopsies of non-

responding melanoma patients treated with checkpoint blockade. In a murine mouse model, the effect of anti-CTLA4 and anti-PD1 treatment was completely abrogated by the addition of glucocorticoid, highlighting the clinical relevance of glucocorticoid signaling modulation for immunotherapy.[61]

In his first part of the talk **Vincenzo Bronte (University of Verona, Italy)** focused on disabled homolog 2 (DAB2), which he and colleagues found to be expressed by tumor-associated macrophages (TAMs) and which exhibited a metastasis-supporting function, as myeloid-specific knock out of Dab2 in mice reduced metastatic burden in the lung. Immunohistochemistry revealed the localization of DAB2-expressing macrophages at the tumor border, which showed protumoral gene signature, characterized by the upregulation of genes involved in M2 polarization and tissue remodeling. Using an inverted cell invasion assay, they discovered that tumor cell invasion was promoted by DAB2, which was found to be YAP/TAZ-dependent, via haptotaxis but not through chemotaxis or trans-endothelial migration. Confirming the relevance for human cancer, Bronte presents DAB2 as a negative prognostic factor in breast cancer patients, additionally showing that genetic deletion improves treatment of metastases with checkpoint blockade.[62] Proceeding with a second part, Bronte first focused on PD-L1$^+$, c-FLIP$^+$, immunosuppressive monocytes with a distinct genetic profile characterized by high pSTAT3 and ARG1, which together with high IL-6 serum levels were associated with poor survival in PDAC patients.[63,64] Following, he described that severe cases of COVID-19 were associated with myeloid-dependent immune suppression loss, marked by low HLA-DR and ARG1 expression,[65] which he supposed could be connected to severe inflammatory responses due to uncontrolled cytokine release. Baricitinib, blocking the JAK/STAT signaling pathway, restored normal lymphocyte counts in blood and decreased levels of pro-inflammatory cytokines, related to the severity of the disease.[66]

Bioinformatics

The improvement of cancer immunotherapies requires new tools that capture accurate picture of the underlying disease and that approach the question how to combine multiple targets. **Ido Amit (Weizmann Institute of Science, Israel)** emphasized that massively parallel single-cell RNA sequencing (MARS-seq) supports a detailed capture of cell-mediated immunity[67] and that the resulting high-resolution maps identify appropriate mouse models that can be used for deeper understanding of the disease. As an example, he showed the validation of a synergistic combination of a new target ("anti-target Y") with anti-PD1 treatment using MARS-seq. Moving on, Amit presented the new generations of single-cell technologies. PIC (physically interacting cells) sequencing is a new technology developed by his lab that combines cell sorting with single-cell RNA-seq and that allows to identify immune cell interactions at the single-cell level.[68] An interesting application of PIC-seq is to decipher the interaction between myeloid cells and T cells in human NSCLC (Non-small-cell lung cancer) tumors. Here, Amit and his lab found CD4$^+$ T helper cells to interact with myeloid cells in tertiary lymphoid structures in the tumor microenvironment while upregulating immune checkpoints. A further example for the new generation of single-cell technology is IN-seq that combines MARS-seq and measurement of intracellular protein activity by intracellular labelling of enzymes.[69] A combined deciphering of immune signatures and metabolic activity can allow the identification of new immune subsets. Using this technology, Amit and his team identified a novel Arg1$^+$ Trem2$^+$ regulatory myeloid (Mreg) cell population, which has an important function in tumor-immune-escape.

Next, **Zlatko Trajanoski (Medical University of Innsbruck, Austria)** accentuated the colorectal cancer paradox which highlights that colorectal cancers are under immunological control but a majority of the patients does not respond to cancer immunotherapy.[70] As previous studies were not successful, he pointed out that new rationales are needed to sensitize these tumors for cancer immunotherapy by targeted drugs. To overcome previous limitations, Trajanoski's lab uses data-driven strategies that consider associations between patient genotypes and immunophenotypes.[71] A very interesting strategy of Trajanoski's lab to support personalized characterization of signalling pathways is to generate organoids from tumors as patient avatars for perturbation studies to elucidate signalling networks in these avatars. Trajanoski showed that these patient-derived organoids develop 3D structures and consist of multiple cell types. Each of the patient avatars are comprehensively characterized by exome and RNA-sequencing and proteomic analyses. Here, Trajanoski emphasized on the importance of proteome studies due to the discrepancies between transcriptomic and proteomic levels. Trajanoski's lab uses these data to extract the individual characteristics of each patient avatar and to design perturbation experiments with e.g. kinase inhibitors. Proteomic and phospho-proteomic profiling of the perturbed organoids is used to decipher the crosstalk between oncogenic and immune evasion pathways. In the end of his talk, Trajanoski highlighted that CyTOF (Cytometry by time of flight) of tumor tissue can be used to reconstruct cell-cell interactions, identifying attracted and avoiding cell compartments.

In the last talk of this session, **Alex Rubinsteyn (University of North Carolina at Chapel Hill, USA)** recapitulated that individualized cancer vaccines aim to direct T cells against mutations. He pointed out that neoantigens are beneficial in comparison with vaccines against non-mutated, shared antigens due to their tumor specificity. While the pillars of neoantigen vaccine design are tumor sequencing, mutation identification, mutation prioritization, and the platform for vaccine delivery, Rubinsteyn focused on embracing the dark matter of antigens in his talk. He emphasized that previous studies focused on identification of single nucleotide variants (SNVs) from short-read based whole exome sequencing (WES) data.[13,38,72,73] As consequence, the set of identified targets was previously underestimated. Further, Rubinsteyn draw attention to disadvantages of short-read WES by highlighted that low-coverage whole genome sequencing (WGS) allows identification of SNVs and INDELs (insertion and deletion) with higher sensitivity in comparison to high-coverage WES sequencing.[74] A further advantage of WGS data is the possibility to identify larger mutations such as larger INDELs and

structural variations. These classes of mutations lead to peptide sequences more dissimilar to self in comparison with SNVs and therefore could be high-quality neoantigen candidates.[75] Rubinsteyn proposed that limitation of short-read data can be overcome by using linked or long-read sequencing. In particular, long read-sequencing could support the identification of mutations that are missed in short-read sequencing data. In the end of his talk, Rubinsteyn introduced a pilot study in which he will analyze the ability of different sequencing technology to support the identification of neoantigens from multiple classes with high confidence. This could allow to broaden the neoantigen target space for individualized cancer vaccines which is especially important in tumor types with low SNV burden, it is planned to use the large mutations identified with long-read sequencing technology in the PANDA-VAC trials (Trial of a Personalized and Adaptive Neoantigen Dose-Adjusted Vaccine Concurrently With Pembrolizumab) (NCT04266730).

Improving immunity

While immunotherapy has improved the prognosis of patients suffering from various kinds of cancer, persistent antitumor immune responses in patients with solid tumors are still scarce.[76] A major cause is the exhaustion of $CD8^+$ T cells in the tumor microenvironment, the result of a co-evolutionary process **Andrea Schietinger (Memorial Sloan Kettering Cancer Center, USA)** and her team are investigating. In an autochthonous liver cancer model (ASTxCre), the group isolated and marked antigen-specific $CD8^+$ T cells for an adoptive transfer into ASTxCre mice before tumor induction to investigate co-evolution of T cells and tumor. Adoptively transferred T cells are reprogrammed into a reversible dysfunctional state at early times after tumor induction and are progressing into a fixed, irreversible dysfunctional state at latter times. Phenotypically they show at this state expression of the exhaustion markers PD1 and LAG accompanied by reduced IFNγ and TNFα expression.[77] The transcriptional regulator TOX is upregulated in dysfunctional intratumoral T cells and is correlated with high exhaustion marker expression in the autochthonous model but also in human tumors. $Tox^{-/-}$ $CD8^+$ T cells surprisingly showed dysfunctionality while preserving a normal phenotype indicating that the functional state and the phenotype are uncoupled.[78] As $Tox^{-/-}$ T cells additionally underwent apoptosis in high levels in the TME, a model of two nodules is proposed in which TOX is a master regulator of exhaustion marker expression leading to T-cell persistence, yet is not responsible for the effector function. In a model that uses varying TCR signal strengths the group showed that high affinity TCRs, whilst being able to kill targets, get exhausted earlier. On the other hand, T cells with low affinity TCRs stay functionally inert whilst being unable to kill. In result, there seems to be a sweet spot of TCR affinity leading to killing but not dysfunctionality that can possibly be used to increase antitumoral immunity.

Another approach of improving antitumoral immunity is the remodeling of the TME by RNA injections examined by the laboratory of **Darrell Irvine (Massachusetts Institute of Technology, USA)**. Inspired by oncolytic viruses, the group is examining transport of self-replicating RNA to the tumor using a carrier that promotes an intrinsic cytotoxicity. Three different lipid nanoparticles, Lipofectamine, DOSPA (both commercially available), and TT3[79] are used to deliver replicon RNA. While non-toxic gene delivery using Lipofectamine and DOSPA shows low efficacy, TT3 shows high transfection levels but also induces immunogenic cell death *in vitro*, which is further amplified by the activity of the replicon RNA. *In vivo*, TT3-encoded reporter RNA targets tumor cells with high affinity and leads to delayed tumor growth. To overturn the TME, a Lumican-Alb-scIL12 fusion protein[80] is used as replicon-RNA encoded payload, which binds to collagen and thus prevents systemic leaking of IL-12. Intratumoral delivery of the LNP-replicon *in vivo* leads to an upregulation of pro-inflammatory genes in the tumor while systemic IFNγ or IL-12 sequestration is prevented. In synergistic treatment of LNP-replicon injection and systemic immunotherapy such as α-PD1 treatment resulted in abscopal responses in different tumor models. The group also showed that the LNP-Replicon treatment leads to a complete rejection upon rechallenge due to the induction of $CD8^+$ memory T cells.

To induce targeted immune cell responses, autologous T cells can be transduced with CARs targeting tumor antigens. Whilst CAR T cells induce responses in liquid tumors, they fail to induce long-lasting effects in solid tumors due to the inhibition in the TME. Further, CAR T cells are losing effector function after antigen loss in liquid tumors, both issues which **Yvonne Chen (University of California, Los Angeles, USA)** and her team are addressing. The group designed an OR-gate CAR being activated after recognition of CD19 or CD20,[81] with high response rates in Phase 1 clinical trials. Successful proof of concept therapies *in vivo* with CARs against CS1 and BCMA indicate the transferability of the method to other types of liquid tumors such as multiple myeloma. If co-treated with α-PD1, the CAR T cells showed dysfunctionality when mice are rechallenged with tumor cells in contrast to mice that were not treated with α-PD1.[82] This indicates that the dysfunctionality might be caused by the addition of α-PD1 in the absence of antigen stimulus. CAR T cells are prone to inhibition by the TME, mostly caused by TGF-β. To tackle this problem, a bispecific CAR has been designed to respond to the soluble version of TGF-ß while also reacting to IL-13 mutein, which shows promising results in first *in vivo* studies.[83–85]

Conclusion

Alberto Mantovani received the CIMT Lifetime Achievement Award for his significant contributions to cancer immunotherapy research. We are looking forward to discussing more advances in the field of cancer immunotherapy hopefully in person at the 19th Annual CIMT Meeting 2022 (Mainz, Germany).

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References

1. Martínez Bedoya D, Dutoit V, Migliorini D. Allogeneic CAR T cells: an alternative to overcome challenges of CAR T cell therapy in glioblastoma. Front Immunol. 2021;12:640082. eng. doi:10.3389/fimmu.2021.640082.
2. Stenger D, Stief TA, Kaeuferle T, Willier S, Rataj F, Schober K, Vick B, Lotfi R, Wagner B, Grünewald TGP, et al. Endogenous TCR promotes in vivo persistence of CD19-CAR-T cells compared to a CRISPR/Cas9-mediated TCR knockout CAR. Blood. 2020;136(12):1407–1418. eng. doi:10.1182/blood.2020005185.
3. Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, Butler K, Rivat C, Wright G, Somana K, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med. 2017;9(374). eng. doi:10.1126/scitranslmed.aaj2013.
4. Jahn L, Hombrink P, Hagedoorn RS, Kester MGD, van der Steen DM, Rodriguez T, Pentcheva-Hoang T, de Ru AH, Schoonakker MP, Meeuwsen MH, et al. TCR-based therapy for multiple myeloma and other B-cell malignancies targeting intracellular transcription factor BOB1. Blood. 2017;129(10):1284–1295. eng. doi:10.1182/blood-2016-09-737536.
5. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78(1):21–33. eng. doi:10.4065/78.1.21.
6. Chan CJ, Smyth MJ, Martinet L. Molecular mechanisms of natural killer cell activation in response to cellular stress. Cell Death Differ. 2014;21(1):5–14. eng. doi:10.1038/cdd.2013.26.
7. Kumari S, Wälchli S, Fallang L-E, Yang W, Lund-Johansen F, Schumacher TN, Olweus J. Alloreactive cytotoxic T cells provide means to decipher the immunopeptidome and reveal a plethora of tumor-associated self-epitopes. Proc Natl Acad Sci U S A. 2014;111(1):403–408. eng. doi:10.1073/pnas.1306549111.
8. Ali M, Giannakopoulou E, Li Y, Lehander M, Virding Culleton S, Yang W, Knetter C, Can Odabasi M, Bollineni RC, Yang X, et al. T cells targeted to TdT kill leukemic lymphoblasts while sparing normal lymphocytes. Nat Biotechnol doi:10.1038/s41587-021-01089-x. 2021. eng.
9. Bartok O, Pataskar A, Nagel R, Laos M, Goldfarb E, Hayoun D, Levy R, Körner P-R, Kreuger IZM, Champagne J, et al. Anti-tumour immunity induces aberrant peptide presentation in melanoma. Nature. 2021;590(7845):332–337. eng. doi:10.1038/s41586-020-03054-1.
10. Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E, Ketelaars SLC, Cheng K, Vervier K, Shental N, et al. Identification of bacteria-derived HLA-bound peptides in melanoma. Nature. 2021;592(7852):138–143. eng. doi:10.1038/s41586-021-03368-8.
11. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, Franci C, Cheung TK, Fritsche J, Weinschenk T, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature. 2014;515(7528):572–576. eng. doi:10.1038/nature14001.
12. Lynn GM, Sedlik C, Baharom F, Zhu Y, Ramirez-Valdez RA, Coble VL, Tobin K, Nichols SR, Itzkowitz Y, Zaidi N, et al. Peptide-TLR-7/8a conjugate vaccines chemically programmed for nanoparticle self-assembly enhance CD8 T-cell immunity to tumor antigens. Nat Biotechnol. 2020;38(3):320–332. eng. doi:10.1038/s41587-019-0390-x.
13. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217–21. eng. doi:10.1038/nature22991.
14. Hu Z, Leet DE, Allesøe RL, Oliveira G, Li S, Luoma AM, Liu J, Forman J, Huang T, Iorgulescu JB, et al. Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma. Nat Med. 2021;27(3):515–525. eng. doi:10.1038/s41591-020-01206-4.
15. Grint DJ, Wing K, Williamson E, McDonald HI, Bhaskaran K, Evans D, Evans SJ, Walker AJ, Hickman G, Nightingale E, et al. Case fatality risk of the SARS-CoV-2 variant of concern B.1.1.7 in England, 16 November to 5 February. Euro Surveill. 2021;26(11). eng. doi:10.2807/1560-7917.ES.2021.26.11.2100256.
16. Davies NG, Jarvis CI, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature. 2021;593(7858):270–74. eng. doi:10.1099/mgen.0.000093.
17. Challen R, Brooks-Pollock E, Read JM, Dyson L, Tsaneva-Atanasova K, Danon L. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study. BMJ. 2021;372:n579. eng. doi:10.1136/bmj.n579.
18. Bager P, Wohlfahrt J, Fonager J, Albertsen M, Yssing Michaelsen T, Holten Møller C, Ethelberg S, Legarth R, Fischer Button MS, Gubbels SM, et al. Increased risk of hospitalisation associated with infection with SARS-CoV-2 Lineage B.1.1.7 in Denmark. SSRN J. 2021. doi:10.2139/ssrn.3792894.
19. Brown JC, Goldhill DH, Zhou J, Peacock TP, Frise R, Goonawardane N, Baillon L, Kugathasan R, Pinto AL, McKay PF, et al. Increased transmission of SARS-CoV-2 lineage B.1.1.7 (VOC 2020212/01) is not accounted for by a replicative advantage in primary airway cells or antibody escape. Cold Spring Harbor Laboratory; 2021. doi:10.1101/2021.02.24.432576.
20. Kissler SM, Fauver JR, Mack C, Tai CG, Breban MI, Watkins AE, Samant RM, Anderson DJ, Ho DD, Metti J, et al. Viral dynamics of SARS-CoV-2 variants in vaccinated and unvaccinated individuals (: medRxiv) doi:10.1101/2021.02.16.21251535. 2021.
21. Lamers MM, Breugem TI, Mykytyn AZ, Wang Y, Groen N, Knoops K, Schipper D, van der Vaart J, Koopman CD, Zhang J, et al. Human organoid systems reveal in vitro correlates of fitness for SARS-CoV-2 B.1.1.7. Cold Spring Harbor Laboratory; 2021. doi:10.1101/2021.05.03.441080.
22. He Z, Ren L, Yang J, Guo L, Feng L, Ma C, Wang X, Leng Z, Tong X, Zhou W, et al. Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study. Lancet. 2021;397(10279):1075–84. eng. doi:10.1016/S0140-6736(21)00238-5.
23. Murhekar MV, Bhatnagar T, Thangaraj JWV, Saravanakumar V, Kumar MS, Selvaraju S, Rade K, Kumar CPG, Sabarinathan R, Turuk A, et al. SARS-CoV-2 seroprevalence among the general population and healthcare workers in India, December 2020-January 2021. Int J Infect Dis. 2021;108:145–55. eng. doi:10.1016/j.ijid.2021.05.040.
24. Yadav PD, Sapkal GN, Abraham P, Ella R, Deshpande G, Patil DY, Nyayanit DA, Gupta N, Sahay RR, Shete AM, et al. Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees. Clin Infect Dis. 2021. eng. doi:10.1093/cid/ciab411.
25. Liu Y, Liu J, Xia H, Zhang X, Zou J, Fontes-Garfias CR, Weaver SC, Swanson KA, Cai H, Sarkar R, et al. BNT162b2-elicited neutralization against new SARS-CoV-2 Spike variants. N Engl J Med. 2021. eng. doi:10.1056/NEJMc2106083.
26. Tarke A, Sidney J, Methot N, Zhang Y, Dan JM, Goodwin B, Rubiro P, Sutherland A, da Silva Antunes R, Frazier A, et al. Negligible impact of SARS-CoV-2 variants on CD4 + and CD8 + T cell reactivity in COVID-19 exposed donors and vaccinees. bioRxiv. 2021. eng. doi:10.1101/2021.02.27.433180.

27. Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Hurtado Del Pozo C, Prosper F, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020;181(4):905–913.e7. eng. doi:10.1016/j.cell.2020.04.004.
28. Monteil V, Dyczynski M, Lauschke VM, Kwon H, Wirnsberger G, Youhanna S, Zhang H, Slutsky AS, Hurtado Del Pozo C, Horn M, et al. Human soluble ACE2 improves the effect of remdesivir in SARS-CoV-2 infection. EMBO Mol Med. 2021;13(1):e13426. eng. doi:10.15252/emmm.202013426.
29. Boczkowski D, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med. 1996;184(2):465–72. eng. doi:10.1084/jem.184.2.465.
30. Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci O, Sahin U. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. Cancer Res. 2010;70(22):9031–40. eng. doi:10.1158/0008-5472.CAN-10-0699.
31. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, Meng M, Fritz D, Vascotto F, Hefesha H, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016;534(7607):396–401. eng. doi:10.1038/nature18300.
32. Holtkamp S, Kreiter S, Selmi A, Simon P, Koslowski M, Huber C, Türeci O, Sahin U. Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. Blood. 2006;108(13):4009–17. eng. doi:10.1182/blood-2006-04-015024.
33. Kreiter S, Selmi A, Diken M, Sebastian M, Osterloh P, Schild H, Huber C, Türeci O, Sahin U. Increased antigen presentation efficiency by coupling antigens to MHC class I trafficking signals. J Immunol. 2008;180(1):309–18. eng. doi:10.4049/jimmunol.180.1.309.
34. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Weissman D. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16(11):1833–40. eng. doi:10.1038/mt.2008.200.
35. Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. 2011;39(21):e142. eng. doi:10.1093/nar/gkr695.
36. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, Maurus D, Schwarck-Kokarakis D, Kuhn AN, Omokoko T, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature. 2020;585(7823):107–12. eng. doi:10.1038/nature23306.
37. Reinhard K, Rengstl B, Oehm P, Michel K, Billmeier A, Hayduk N, Klein O, Kuna K, Ouchan Y, Wöll S, et al. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science. 2020;367(6476):446–53. eng. doi:10.1126/science.aay5967.
38. Sahin U, Derhovanessian E, Miller M, Kloke B-P, Simon P, Löwer M, Bukur V, Tadmor AD, Luxemburger U, Schrörs B, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222–26. eng. doi:10.1038/nature23003.
39. Krienke C, Kolb L, Diken E, Streuber M, Kirchhoff S, Bukur T, Akilli-Öztürk Ö, Kranz LM, Berger H, Petschenka J, et al. A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. Science. 2021;371(6525):145–53. eng. doi:10.1126/science.aay3638.
40. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Raabe V, Bailey R, Swanson KA, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;586(7830):589–93. eng. doi:10.1038/s41467-020-17892-0.
41. Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med. 2020;383(25):2439–50. eng. doi:10.1056/NEJMoa2027906.
42. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603–15. eng. doi:10.1056/NEJMoa2034577.
43. Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M, Kranz LM, Walzer KC, Hein S, Güler A, et al. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. Nature. 2021;592(7853):283–89. eng. doi:10.1038/s41586-021-03275-y.
44. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20. eng. doi:10.1056/NEJMoa1500596.
45. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–13. eng. doi:10.1126/science.aan6733.
46. van den Bulk J, Verdegaal EME, Ruano D, Ijsselsteijn ME, Visser M, van der Breggen R, Duhen T, van der Ploeg M, de Vries NL, Oosting J, et al. Neoantigen-specific immunity in low mutation burden colorectal cancers of the consensus molecular subtype 4. Genome Med. 2019;11(1):87. eng. doi:10.1186/s13073-019-0697-8.
47. van den Bulk J, de Miranda NFCC, Ten Dijke P. Therapeutic targeting of TGF-β in cancer: hacking a master switch of immune suppression. Clin Sci (Lond). 2021;135(1):35–52. eng. doi:10.1042/CS20201236.
48. de Vries NL, van Unen V, Ijsselsteijn ME, Abdelaal T, van der Breggen R, Farina Sarasqueta A, Mahfouz A, Peeters KCMJ, Höllt T, Lelieveldt BPF, et al. High-dimensional cytometric analysis of colorectal cancer reveals novel mediators of antitumour immunity. Gut. 2020;69(4):691–703. eng. doi:10.1136/gutjnl-2019-318672.
49. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: can Treg cells be a new therapeutic target? Cancer Sci. 2019;110(7):2080–89. eng. doi:10.1111/cas.14069.
50. Wang H, Franco F, Tsui Y-C, Xie X, Trefny MP, Zappasodi R, Mohmood SR, Fernández-García J, Tsai C-H, Schulze I, et al. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. Nat Immunol. 2020;21(3):298–308. eng. doi:10.1038/s41590-019-0589-5.
51. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob -J-J, Cowey CL, Lao CD, Wagstaff J, Schadendorf D, Ferrucci PF, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2017;377(14):1345–56. eng. doi:10.1056/NEJMoa1709684.
52. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, Johansson I, Phung B, Harbst K, Vallon-Christersson J, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. 2020;577(7791):561–65. eng. doi:10.1073/pnas.091062498.
53. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020;577(7791):549–55. eng. doi:10.1038/s41467-017-00608-2.
54. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359(6371):91–97. eng. doi:10.1126/science.aan3706.
55. Cascone T, William WN, Weissferdt A, Leung CH, Lin HY, Pataer A, Godoy MCB, Carter BW, Federico L, Reuben A, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in operable non-small cell lung cancer: the phase 2 randomized NEOSTAR trial. Nat Med. 2021;27(3):504–14. eng. doi:10.1093/nar/gks1219.
56. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre M-L, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084–89. eng. doi:10.1126/science.aac4255.

57. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CPM, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350(6264):1079–84. eng. doi:10.1126/science.aad1329.
58. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin J-M, Morrison RM, Deblasio RN, Menna C, Ding Q, Pagliano O, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science. 2021;371(6529):595–602. eng. doi:10.1126/science.abf3363.
59. Shaikh FY, White JR, Gills JJ, Hakozaki T, Richard C, Routy B, Okuma Y, Usyk M, Pandey A, Weber JS, et al. A uniform computational approach improved on existing pipelines to reveal microbiome biomarkers of nonresponse to immune checkpoint inhibitors. Clin Cancer Res. 2021;27(9):2571–83. eng. doi:10.1158/1078-0432.CCR-20-4834.
60. Welters MJP, Ma W, Santegoets SJAM, Goedemans R, Ehsan I, Jordanova ES, van Ham VJ, van Unen V, Koning F, van Egmond SI, et al. Intratumoral HPV16-specific T cells constitute a Type I-oriented tumor microenvironment to improve survival in HPV16-driven oropharyngeal cancer. Clin Cancer Res. 2018;24(3):634–47. eng. doi:10.1158/1078-0432.CCR-17-2140.
61. Acharya N, Madi A, Zhang H, Klapholz M, Escobar G, Dulberg S, Christian E, Ferreira M, Dixon KO, Fell G, et al. Endogenous glucocorticoid signaling regulates CD8+ T cell differentiation and development of dysfunction in the tumor microenvironment. Immunity. 2020;53(3):658–671.e6. eng. doi:10.1016/j.immuni.2020.08.005.
62. Marigo I, Trovato R, Hofer F, Ingangi V, Desantis G, Leone K, de Sanctis F, Ugel S, Canè S, Simonelli A, et al. Disabled homolog 2 controls prometastatic activity of tumor-associated macrophages. Cancer Discov. 2020;10(11):1758–73. eng. doi:10.1158/2159-8290.CD-20-0036.
63. Fiore A, Ugel S, de Sanctis F, Sandri S, Fracasso G, Trovato R, Sartoris S, Solito S, Mandruzzato S, Vascotto F, et al. Induction of immunosuppressive functions and NF-κB by FLIP in monocytes. Nat Commun. 2018;9(1):5193. eng. doi:10.1038/s41467-018-07654-4.
64. Trovato R, Fiore A, Sartori S, Canè S, Giugno R, Cascione L, Paiella S, Salvia R, de Sanctis F, Poffe O, et al. Immunosuppression by monocytic myeloid-derived suppressor cells in patients with pancreatic ductal carcinoma is orchestrated by STAT3. J Immunother Cancer. 2019;7(1):255. eng. doi:10.1186/s40425-019-0734-6.
65. Bost P, de Sanctis F, Canè S, Ugel S, Donadello K, Castellucci M, Eyal D, Fiore A, Anselmi C, Barouni RM, et al. Deciphering the state of immune silence in fatal COVID-19 patients. Nat Commun. 2021;12(1):1428. eng. doi:10.1038/s41467-021-21702-6.
66. Bronte V, Ugel S, Tinazzi E, Vella A, de Sanctis F, Canè S, Batani V, Trovato R, Fiore A, Petrova V, et al. Baricitinib restrains the immune dysregulation in patients with severe COVID-19. J Clin Invest. 2020;130(12):6409–16. eng. doi:10.1172/JCI141772.
67. Jaitin DA, Kenigsberg E, Keren-Shaul H, Elefant N, Paul F, Zaretsky I, Mildner A, Cohen N, Jung S, Tanay A, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. Science. 2014;343(6172):776–79. eng. doi:10.1126/science.1247651.
68. Giladi A, Cohen M, Medaglia C, Baran Y, Li B, Zada M, Bost P, Blecher-Gonen R, Salame T-M, Mayer JU, et al. Dissecting cellular crosstalk by sequencing physically interacting cells. Nat Biotechnol. 2020;38(5):629–37. eng. doi:10.1038/s41587-020-0442-2.
69. Katzenelenbogen Y, Sheban F, Yalin A, Yofe I, Svetlichnyy D, Jaitin DA, Bornstein C, Moshe A, Keren-Shaul H, Cohen M, et al. Coupled scRNA-Seq and intracellular protein activity reveal an immunosuppressive role of TREM2 in cancer. Cell. 2020;182(4):872–885.e19. eng. doi:10.1016/j.cell.2020.06.032.
70. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5795):1960–64. eng. doi:10.1126/science.1129139.
71. Angelova M, Charoentong P, Hackl H, Fischer ML, Snajder R, Krogsdam AM, Waldner MJ, Bindea G, Mlecnik B, Galon J, et al. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy. Genome Biol. 2015;16:64. eng. doi:10.1186/s13059-015-0620-6.
72. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, et al. A Phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell. 2020;183(2):347–362.e24. eng. doi:10.1016/j.cell.2020.08.053.
73. Rubinsteyn A, Kodysh J, Hodes I, Mondet S, Aksoy BA, Finnigan JP, Bhardwaj N, Hammerbacher J. Computational pipeline for the PGV-001 neoantigen vaccine trial. Front Immunol. 2017;8:1807. eng. doi:10.3389/fimmu.2017.01807.
74. Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, Antipenko A, Shang L, Boisson B, Casanova J-L, Abel L. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proc Natl Acad Sci U S A. 2015;112(17):5473–78. eng. doi:10.1073/pnas.1418631112.
75. McGranahan N, Swanton C. Neoantigen quality, not quantity. Sci Transl Med. 2019;11(506). eng. doi:10.1126/scitranslmed.aax7918.
76. Zhang J-Y, Yan -Y-Y, Li -J-J, Adhikari R, Fu L-W. PD-1/PD-L1 based combinational cancer therapy: icing on the cake. Front Pharmacol. 2020;11:722. eng. doi:10.3389/fphar.2020.00722.
77. Philip M, Fairchild L, Sun L, Horste EL, Camara S, Shakiba M, Scott AC, Viale A, Lauer P, Merghoub T, et al. Chromatin states define tumour-specific T cell dysfunction and reprogramming. Nature. 2017;545(7655):452–56. eng. doi:10.1038/nature22367.
78. Scott AC, Dündar F, Zumbo P, Chandran SS, Klebanoff CA, Shakiba M, Trivedi P, Menocal L, Appleby H, Camara S, et al. TOX is a critical regulator of tumour-specific T cell differentiation. Nature. 2019;571(7764):270–74. eng. doi:10.1038/s41586-019-1324-y.
79. Luo X, Li B, Zhang X, Zhao W, Bratasz A, Deng B, McComb DW, Dong Y. Dual-functional lipid-like nanoparticles for delivery of mRNA and MRI contrast agents. Nanoscale. 2017;9(4):1575–79. eng. doi:10.1039/c6nr08496f.
80. Momin N, Mehta NK, Bennett NR, Ma L, Palmeri JR, Chinn MM, Lutz EA, Kang B, Irvine DJ, Spranger S, et al. Anchoring of intratumorally administered cytokines to collagen safely potentiates systemic cancer immunotherapy. Sci Transl Med. 2019;11(498). eng. doi:10.1126/scitranslmed.aaw2614.
81. Zah E, Lin M-Y, Silva-Benedict A, Jensen MC, Chen YY. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells. Cancer Immunol Res. 2016;4(6):498–508. eng. doi:10.1158/2326-6066.CIR-15-0231.
82. Zah E, Nam E, Bhuvan V, Tran U, Ji BY, Gosliner SB, Wang X, Brown CE, Chen YY. Systematically optimized BCMA/CS1 bispecific CAR-T cells robustly control heterogeneous multiple myeloma. Nat Commun. 2020;11(1):2283. eng. doi:10.1038/s41467-020-16160-5.
83. Chang ZL, Lorenzini MH, Chen X, Tran U, Bangayan NJ, Chen YY. Rewiring T-cell responses to soluble factors with chimeric antigen receptors. Nat Chem Biol. 2018;14(3):317–24. eng. doi:10.1038/nchembio.2565.
84. Hou AJ, Chang ZL, Lorenzini MH, Zah E, Chen YY. TGF-β-responsive CAR-T cells promote anti-tumor immune function. Bioeng Transl Med. 2018;3(2):75–86. eng. doi:10.1002/btm2.10097.
85. Chang ZL, Hou AJ, Chen YY. Engineering primary T cells with chimeric antigen receptors for rewired responses to soluble ligands. Nat Protoc. 2020;15(4):1507–24. eng. doi:10.1038/s41596-020-0294-8.