

However, the mechanisms contributing to the therapeutic success have not been entirely uncovered by now. Here we focus on the impact of PD-1/CTLA-4-blocking antibodies on regulatory T cells (Tregs), which are known to be involved in tumor immune evasion in many cancer types.

**Methods** To evaluate how Tregs are affected by anti-PD-1/CTLA-4 therapy, we used a MYC-transgenic mouse model of spontaneously arising B-cell lymphoma, which can be effectively treated by immune checkpoint inhibition. Data were acquired by flow cytometry.

**Results** As earlier shown, Tregs were involved in immune escape of MYC tumors. The Treg to effector T cell (Teff) ratio was elevated within the CD4-positive cell compartment. Tumor-infiltrating Tregs were predominantly thymic Tregs, which recognized overexpressed tumor-derived self-peptides in an MHC class II-restricted manner and showed upregulated expression of activation markers, Foxp3, CD25 and IL-10. To examine whether these phenotypic alterations correlated with the immunosuppressive capability of Tregs, an in vitro suppression assay was established. In this setting, MYC Tregs turned out to suppress proliferation and IFN- $\gamma$  release of Teff cells more effectively than wildtype Tregs. The suppression observed in vitro was mediated by cell contacts and IL-10. Further suppressive mechanisms are likely to play a role, such as competition for MHC-II ligands and a consumption of IL-2. To investigate if immune checkpoint blockade interferes with these Treg-dependent immunosuppressive pathways, MYC mice were treated with a combination of anti-PD-1 and anti-CTLA-4 antibodies. Tregs from treated MYC mice showed decreased expression of CD69, CD137, Foxp3, CD25 and IL-10 as compared to Tregs from untreated MYC mice. This correlated with a lower suppressive capacity of Tregs from treated animals in the in vitro suppression assay.

**Conclusions** Taken together, the data show that immune checkpoint blockade impairs the development of the suppressive phenotype of intratumoral Tregs. Thus, apart from the initially described Teff reactivation, other mechanisms are also relevant for unfolding the therapeutic effect of immune checkpoint inhibitors.

**Ethics Approval** All animal experiments were approved by Regierung von Oberbayern, approval number 55.2-1-54.

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#### QUANTITATIVE CELL-BASED BIOASSAYS TO ADVANCE IMMUNOTHERAPY PROGRAMS TARGETING IMMUNE CHECKPOINT RECEPTORS

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**Background** The human immune system is comprised of a complex network of immune checkpoint receptors that are promising new immunotherapy targets for the treatment of a variety of cancers and autoimmune disorders. The Nobel prize for Medicine in 2018 was awarded for seminal studies on the role of immune checkpoint targets in T cell activation and furthermore, combining different strategies to release the brakes on the immune system with the aim of eliminating tumor cells even more efficiently. Immunotherapies designed to block co-inhibitory receptors (e.g. PD-1, CTLA-4, TIGIT) are showing unprecedented efficacy in the treatment of cancer. However, not all patients and tumor types respond to this approach.

This has resulted in broadening of immunotherapy research programs to target additional co-inhibitory (e.g. LAG-3, TIM-3) and co-stimulatory (e.g. 4-1BB, GITR, OX40, ICOS) receptors individually and in combination.

A major challenge in the development of antibody-based biologics is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable and fail to yield data required for drug development in a quality-controlled environment.

**Methods** To address the need of access to a robust and reliable functional assay for immunotherapy drug development programs, we have developed a suite of cell-based functional bioassays to interrogate modulation of immune checkpoint receptors individually (e.g. PD-1, CTLA-4, LAG-3, TIM-3, GITR, 4-1BB, OX40, CD40) and in combination (e.g. PD-1 + CTLA-4, PD-1 + 4-1BB). These assays consist of stable cell lines that express luciferase reporters driven by response elements under the precise control of mechanistically relevant intracellular signals. Thus, the bioassays reflect mechanisms of action for the drug candidates designed for each immune checkpoint receptor and demonstrate high specificity, sensitivity and reproducibility. Here we describe the application of MoA-based immune checkpoint receptor bioassays as tools for biologics drug discovery, development, potency and stability studies.

**Results** We demonstrate that these bioassays measure response and inhibition with blocking drugs or potency changes from stressed samples.

**Conclusions** In summary, these reporter-based bioassays provide valuable tools for the development, stability testing, and potency determination in the manufacture of biologics that are targeted to immune checkpoint.

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#### VALIDATION OF THE COMBINATORIAL EFFECT OF BLINATUMOMAB AND NIVOLUMAB IN CANCER THERAPY

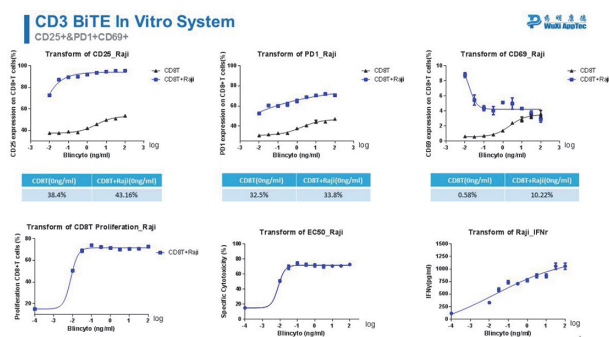
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**Background** Cancer immunotherapies, including immune checkpoint inhibitors, CAR-T, cancer vaccines and bispecific antibodies, have been brought to spot light in recent years as several therapeutic strategies targeting the immune system have produced exciting clinical results. Bispecific antibody typically play dual roles in blocking the immune checkpoint and redirecting/re-boosting the function of the immune effector cells. Blinatumomab belongs to CD3 bispecific T cell engager (CD3 BiTE), which was engineered to harbor two arms binding with CD3 and CD19 simultaneously and direct CD8+ T cells to specifically recognize CD19 positive lymphoma cells to execute cytotoxicity. Approval of Blinatumomab for patients with relapse/refractory B cell acute lymphoblastic leukemia (ALL) has driven remarkable increase in combination studies of Blinatumomab with other immunotherapies such as checkpoint inhibitors.

**Methods** In this study, we developed CD8+ T cytotoxic system targeting different B lymphoma cell line and fully validated the function of Blinatumomab in promoting target tumor cell lysis by primary CD8+ T cells (figure 1). In addition, we established a mixed lymphocyte and tumor system to

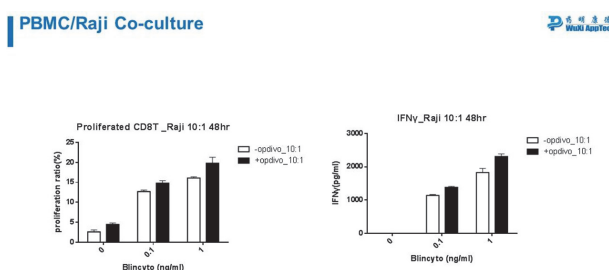
mimic physiological TME to dissect the combinational role of Nivolumab and Blinatumomab (figure 2).

**Results** The result suggest that combinatory therapy is highly depend on the dosage of Blinatumomab and also T cell number in the TME, which might give an instruction for ongoing clinical trial design. Finally, we have employed humanized mouse models bearing Raji or Daudi tumor cells to further validate this combination treatment in vivo. Both In-vivo and In-vitro data support that Blinatumomab is dominant in activating T cell and Nivolumab can only exhibit synergistic effect under suboptimal dosage of Blinatumomab.



**Abstract 781 Figure 1** Establishment of In vitro co-culture system for CD3 BiTE

establish in vitro human PBMC based system to validate CD3 BiTE function



**Abstract 781 Figure 2** Opdivo and CD3 BiTE Combination  
Opdivo could further promote T cell activation under the treatment of CD3 BiTE

**Conclusions** Successfully establish in vitro system to evaluate the function of CD3 BiTE and also take advantage of MLR/tumor co-culture system to demonstrate PD1 antibody could further promote T cell activation under appropriate dosage of CD3 BiTE.

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## 782 ALDH1A INHIBITION AS ADJUVANT TO OVARIAN CANCER IMMUNOTHERAPY

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**Background** Despite some recent advances, new therapeutic approaches for ovarian cancer (OvCa), the 5th leading cause

of cancer deaths in women, are clearly needed. Aldehyde dehydrogenase-1A (ALDH1A) enzymes represent a novel therapeutic target for OvCa. ALDH1A is upregulated in OvCa initiating cells and mediate the biosynthesis of retinoic acid (RA) to regulate numerous cellular processes, including proliferation, metastasis, and chemotherapy resistance. We recently identified novel pan-ALDH1A family inhibitors (ALDHi) that induce necroptosis in OvCa stem-like cells and synergize with chemotherapy, leading to tumor eradication in vivo. Here, we hypothesize that, in addition to controlling tumor progression, ALDHi trigger immunogenic cell death (ICD) via necroptosis and can potentiate anti-tumor immunity.

**Methods** We performed RNA-Seq on four human OvCa cell lines (A2780, CAOV-3, OVCAR-5, OVSAHO) treated for 8 hours with two different ALDHi. To measure the impact on T cell immunity we performed flow cytometry to measure cell proliferation assays and CD4 naïve differentiation into Th1/Th17/Treg subsets. Molecular targets in the RA pathway were confirmed by western blot.

**Results** ALDHi triggered significant changes in (i) ER stress unfolded protein response and regulators of the ER stress response, such as ATF4 and EIF2aK3 (PERK), (ii) inflammatory pathways, (iii) cell death, survival, and (iv) gene transcription-RAR signaling. Treatment of cancer cell lines with ALDHi induced expression of Phospho-eIF2 $\alpha$ , a marker for the ICD, along with increased expression of ATF3 and ATF4, and calreticulin, suggesting cancer cells undergoing ICD.

Using polyclonal stimulation of murine splenocytes and human PBMC, we observed that ALDHi promote T cell proliferation, especially of CD8 T cells. Furthermore, exposure of naïve CD4 cells to Th1 and Treg differentiation conditions leads to increased production of INF $\gamma$  and reduced number of Foxp3+ iTregs, respectively. Further, in a co-culture of iTreg and stimulated splenocyte, ALDHi treatment diminishes the iTreg's capacity to induce immune suppression. Ex vivo treatment of ovarian cancer ascites cells with various ALDHi leads to significant decrease of CD14+ cells, an effect associated with downregulation of NR4A1 (NUR77), a nuclear receptor that interacts RAR/RXR, downstream of RA signaling.

**Conclusions** ALDHi induce immunogenic cell death in cancer cells. Immune cells respond to ALDHi in a cell specific manner. ALDHi support CD8 T cell proliferation and CD4 Th1 induction, while inhibiting iTregs. Exposure to ALDHi leads to downregulation of NR4A1 and reduction in suppressive macrophage numbers. Our results support the use of ALDHi as immune modulators in ovarian cancer and adjuvants to immunotherapy.

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## 785 ESTROGEN-DEPRIVATION PROMOTES TH1 POLARIZATION OF TUMOR-ASSOCIATED T CELLS IN A MOUSE MODEL OF HIGH GRADE SEROUS OVARIAN CANCER

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**Background** Immunotherapy has achieved long-term survival in patients with melanoma and other tumors, introducing a new paradigm in cancer treatment. Differential outcomes among men and women receiving immune checkpoint inhibitors implicate sex steroids as modulators of treatment response. Estrogen signaling has a profound impact on T cell function