**4MO** Preclinical evaluation of novel CDK4/6 inhibitor GLR2007 in breast and lung cancer models

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**Background:** Cyclin-dependent kinases (CDKs) such as CDK4/6 are essential in regulating the cell cycle, which is disrupted in many cancers. Currently marketed CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib have shown preclinical efficacy in solid tumors including breast cancer and non-small cell lung cancer. GLR2007 is an investigational CDK4/6 inhibitor with potential to treat advanced solid tumors. In vitro and in vivo antitumor effects of GLR2007 were investigated in breast and lung cancer cell line preclinical models.

**Methods:** In vitro proliferation inhibition was evaluated through live cell counts in 7 human and murine breast cancer cell lines and 21 human lung cancer cell lines after culture for 72 h with 0.01–10.00 nM GLR2007 or 1.5–10.00 nM abemaciclib, reported as half maximal inhibitory concentration (IC₅₀).

**Results:** GLR2007 inhibited proliferation at lower IC₅₀ values compared to abemaciclib in 5 breast cancer cell lines (IC₅₀ fold difference range = 0.08–0.92; median = 0.33) and in 20 lung cancer cell lines (IC₅₀ fold difference range = 0.03–0.99; median = 0.39). In MCF-7 breast xenografts, compared to vehicle control, 50 mg/kg GLR2007 induced 49.6% tumor growth inhibition (TGI) (P < 0.001) in mice treated for 21 days, and 81.4% TGI (P = 0.037) on day 25 in mice treated for 28 days. In lung cancer subcutaneous xenograft models, compared to vehicle control, 50 mg/kg GLR2007 induced 68.9% TGI (P < 0.001) on day 16 in mice implanted with NCI-H2228 cells and treated for 22 days, and 33.9% TGI (P = 0.003) on day 34 in mice implanted with NCI-H2228 cells and treated for 28 days.

**Conclusions:** In a number of tumor cell lines, GLR2007 inhibited proliferation at lower IC₅₀ values compared to abemaciclib. GLR2007 demonstrated significant antitumor efficacy in xenograft models compared to vehicle controls. These preclinical studies demonstrated the potential of GLR2007 as a novel CDK4/6 inhibitor for the treatment of breast and lung cancer.

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**5MO** CDK4/6 blockade is as effective as immune-checkpoint inhibition in tumor growth control of Mlh1−/− and Msh2loxP/loxP villin-Cre mice

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**Background:** Mismatch-repair deficiency (dMMR) is a hallmark of Lynch syndrome-associated cancers, often resulting from inactivating mutations in MLH1 or MSH2. These tumors have a high likelihood of responding to immune checkpoint inhibitors (ICIs). Still, intrinsic or acquired resistance mechanisms impair patients' outcomes. Here, we compared the therapeutic potential of an anti-PD-1 inhibitor with the CDK4/6 inhibitor abemaciclib in two preclinical mouse models of dMMR-driven carcinogenesis.

**Methods:** In this ongoing trial, Mlh1−/− or Msh2loxP/loxP Villin-Cre mice with gastrointestinal tumors were either treated with anti-PD-L1 monoclonal antibody (clone: 66L1, 2.5 mg/kg bw i.p., q2wwx3) or abemaciclib (75 mg/kg bw p.o., q1wwx). Control mice received the isotype (anti-igG1 2.5 mg/kg bw i.p., q2wwx3) or were left untreated. Blood phenotyping was performed regularly. The tumor microenvironment was studied by immunofluorescence.

**Results:** Both therapies prolonged overall survival of mice significantly: Mlh1−/−: 9.1 wks (abemaciclib) vs. 3.5 wks (control); Msh2loxP/loxP Villin-Cre: 6.0 wks (66L1, ongoing) and 8.2 wks (abemaciclib, ongoing) vs. 1.0 wk (control). One Mlh1−/− mouse received complete remission upon abemaciclib, while anti-PD-L1 therapy primarily induced stable disease at best (PET/CT). Therapeutic effects of abemaciclib were accompanied by increased numbers of tumor-infiltrating CD4⁺CD8⁺ T-cells and lower numbers of M2-macrophages. Blood phenotyping revealed PD-L1 upregulation under abemaciclib therapy.

Conclusions: While ICI-based therapies are effective and FDA approved for dMMR cancer, abemaciclib constitutes a promising alternative therapy option. The strong immune stimulation upon abemaciclib treatment renders this compound ideal for ICI refractory or intrinsically resistant tumors.

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**6MO** PCSK9 inhibitor evolocumab reduces cardiotoxicity and inflammation induced by doxorubicin-trastuzumab sequential treatment through MyD88/NF-κB/mTORC1 pathways

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**Background:** Inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as a novel therapy to treat hypercholesterolemia and related cardiovascular diseases. Evolocumab, a PCSK9 inhibitor, reduced the risk of cardiovascular events in patients with atherosclerotic cardiovascular diseases when added to maximally tolerated statin therapy (i.e. ezetimibe), and recent data from the ODYSSEY OUTCOMES trial indicate that abemaciclib added to maximally tolerated statin therapy (i.e. other lipid-lowering drugs) reduces the risk of cardiovascular events in patients with a recent acute coronary syndrome.

**Methods:** Human fetal cardiomyocytes (HFC cell line) were exposed to subclinical concentration of doxorubicin, trastuzumab, sequential treatment of both (all 100 nM), alone or in combination with evolocumab (50 nM) for 48 h. After the incubation period, we performed the following tests: determination of cell viability, through analysis of mitochondrial dehydrogenase activity, study of lipid peroxidation (quantifying cellular Malondialdehyde and 4-hydroxynonenal), intracellular Ca²⁺ homeostasis. Moreover, pro-inflammatory studied were also performed (activation of NFκB, inflammation; expression of TNFα/MYD88; mTORC1 FoxO1/3a, transcriptional activation of p65/NF-κB and secretion of cytokines involved in cardiotoxicity (Interleukins 1β, 6, 8).

**Results:** Evolocumab co-incubated with doxorubicin alone or in sequence with trastuzumab exerts cardioprotective effects, enhancing cell viability of 35-43% compared to untreated cells (p < 0.05 for all); Evolocumab reduced significantly the cardiotoxicity through MyD88/NF-κB/cytokines axis and mTORC1 FoxO1/3a mediated mechanisms.

**Conclusions:** We demonstrated, for the first time, that the PCSK9 inhibitor evolocumab exerts direct effects in cardiomyocytes during doxorubicin and trastuzumab exposure turning on a new light on its possible use in cancer patients.

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**7MO** Effect of anti-CTLA-4 immunotherapy on lymphocyte subset and activation profiles and clonal composition on the B16F0 mouse melanoma model

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**Background:** Among most promising strategies for cancer immunotherapy is immune checkpoint blockade. However, the remarkable responses to the therapy are currently limited to a minority of patients and indications, highlighting the importance of understanding of immune mechanisms. The purpose of this study was to investigate the effect of anti-CTLA-4 immunotherapy on lymphocyte subset and activation profiles and clonal composition on the B16F0 mouse melanoma model.

**Methods:** The experiments were carried out on C57BL/6 mice bearing B16F0 mouse melanoma. Mice were treated with 250 μg anti-CTLA4 (Bio X Cell, USA). T-lymphocytes were obtained from tumor or lymphatic nodules (LN), analyzed by flow cytometry using a FACSAria III cell sorter, sorted and evaluated by RNA- and TCR (T cell receptor)-seq.
Results: We identified that Th CD4 subset appeared to be primary target of CTLA-4 therapy. The effect consisted of increased ratio of ThCD8 and skew of Th cells from IFNy-secreting towards proliferating cells within the tumor. Analysis of major lymphocyte subsets from LN revealed an increased percentage of activated CD69+/CD25+ cells from Th, CD8+ and B cells and IFNγ secretion by Th. We found that the number of TCR clusters significantly increased in Th after therapy. This indicates proliferation of certain Th clones, yet with unknown specificities. To identify the antigen specificity of these clones we immunized mice with melanoma peptide neo-antigens and harvested T-cells from LN. Then T-cells were cocultured with APCs loaded with different melanoma peptides. We identified up to 75% CD69+/CD25+ activated T-cells to the specific peptides. We detected clones of TCR, which were found with a high frequency in cells reactivated to specific peptides and were not found in the control. Now the data set is in progress. Future experiments will be directed to compare B16F0-specific TCRs with proliferative clones after anti-CTLA4 blockade.

Conclusions: We found that anti-CTLA-4 immunotherapy leads to activation and clonal expansion of lymphocytes with similar TCRs. For detailed analysis, we developed the approach to identify the TCR specificity to specific B16F0 peptides.

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8P The characterization of tumors associated with the antitumor activity of lenvatinib plus anti-PD-1 antibody combination therapy in a mouse syngeneic model panel

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Background: Lenvatinib is a multiple receptor tyrosine kinase inhibitor targeting mainly vascular endothelial growth factor and fibroblast growth factor receptors. We also reported that lenvatinib has immunomodulatory activity that contributes to the antitumor activity of lenvatinib and enhances the antitumor activity in combination treatment with anti-PD-1 antibody (anti-PD-1). In this study, we investigated the characters of tumors associated with the antitumor activity of the lenvatinib plus anti-PD-1 combination treatment.

Methods: We evaluated the antitumor activities of lenvatinib (10 mg/kg, Q.D.), anti-PD-1 (200mg/head, twice weekly), and their combination in 12 mouse syngeneic tumor models. To define the characteristics of tumors, we conducted RNA-seq analysis to examine T cell inflamed GEP (GEP) score using mouse tumors before treatment. We also stained CD31 in the same tumors and calculated micro-vessel density (MVD) score by dividing CD31 positive blood vessels number by tumor area.

Then, the correlation analysis among gene expression levels of GEP, IHC-based MVD expression is increased specifically in Th after therapy. This indicates reaction between tetraine and TCO moiety.

Results: In canines, the highest non-severely toxic dose of SQ3370 was found to be 8.95 times higher when compared to the conventional dose of Dox. There were minimal and reversible systemic adverse events, with no evidence of cardiotoxicity. The PK analysis in dogs showed that SQ3370 efficiently captures SQP33 from circulation and releases active Dox throughout the treatment period. In the absence of SQ70, SQP33 was found to be stable and did not spontaneously activate. In mice, at least 50% of the SQ70 remains at the injection site for 2-4 weeks, and plasma levels of SQP33 and Dox are similar across SQ70 injection locations. SQ3370 elicited dose-dependent antitumor responses in three syngeneic dual tumor models.

Conclusions: SQ3370 enables higher concentrations of the active drug at the tumor site and minimizes systemic adverse effects associated with conventional chemotherapy. The CAPAC Platform represents a new therapeutic modality to treat solid tumors by using a drug with known efficacy, such as Dox, and expanding its pharmacological capabilities.

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10P DS-6000a, a novel CDH6-targeting antibody-drug conjugate with a novel DNA topoisomerase I inhibitor DXd, demonstrates potent antitumor activity in preclinical models

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Background: Human Cadherin 6 (CDH6) is a single transmembrane protein consisting of 790 amino acids classified in the type 2 cadherin family. Human CDH6 is specifically expressed in the brain and kidneys during the development phase and has been reported to systemically decrease CDH6 expression in the adult body. CDH6 expression is increased specifically in renal cell carcinoma (RCC) and ovarian cancer (OVC). Therefore, CDH6 could be an attractive target for cancer therapy. We created DS-6000a, a CDH6-targeting antibody-drug conjugate (ADC) using an enzymatically cleavable tetratetrapeptide-based linker, and a high drug-to-antibody ratio (DAR 7 to 8) with a novel DNA topoisomerase I inhibitor (DXd). In this study, the pharmacological activity and the mechanism of action of DS-6000a were evaluated in preclinical in vitro and in vivo models.

Methods: CDH6 expression was assessed by immunohistochemistry and FCN analysis. Induction of DNA damage and apoptosis to tumor cells by DXd released from DS-6000a were assessed by western blot. In vitro cell growth inhibitory and in vivo antitumor activities of DS-6000a were evaluated using CDH6-high and -low RCC and OVC cell lines, xenograft mouse models and patient derived xenograft (PDX) models.

Results: CDH6 is highly expressed in RCC and OVC patient samples. DS-6000a demonstrated in vitro cell growth inhibitory activity in CDH6-high tumor cells, but not in CDH6-low tumor cells. DNA damage and apoptosis were induced in CDH6-high tumor cells after the in vitro treatment with DXd and DS-6000a, but not with isotype control IgG ADC. DS-6000a exhibited strong antitumor activity with tumor regression in CDH6-high cell lines in mouse xenograft models. DS-6000a also showed high efficacy against PDX models.

Conclusions: Based on these preclinical results, DS-6000a could provide a valuable therapy with a potential benefit in CDH6-expressing cancers at the clinical setting.