of Philadelphia fusion chromosome, coding for BCR-ABL1 oncoprotein. The life-long treatment relies on using tyrosine kinase inhibitors (TKIs). In some cases, patients develop point mutations, leading to resistance to TKIs treatment, nearly in 2%. Allogeneic stem cell transplantation is the possible solution for these individuals in late stages of CML with success cure rate only approximately at 40%.¹ Based on this funding new solutions for treating cancer with genetic etiology are considered. CRISPR/Cas system, composed of guide RNA, targeting endonuclease Cas9 to specific target genomic region has been used before to mediate breakage of Philadelphia chromosome at the site of oncogenic translocation, although at lower efficiency.²

Materials and Methods K562 cells, model for Philadelphia chromosome positive cells, were used. Constructs, expressing BCR-ABL1 targeting gRNA and Cas9, tethered via coiled-coil forming peptides to *E.coli* exonuclease EXOIII, were nucleofected into target cells. T7E1 assay to detect genome modifications was carried out. TUNEL assay, FACS analysis and bioluminescence measurement were used for cell death determination. SCID mice were used for a subcutaneous K562 cancer model.

Results Our strategy was to couple Cas9 to the exonuclease to promote large deletion at the target site. Of the different exonucleases tested, the EXOIII exhibited the best performance in terms of deletion formation. To improve the rate of deletion genetic lesions, we connected Cas9 and EXOIII via coiled-coil forming peptides, bringing the two enzymes into close proximity (CRISPR-EXO). This resulted in an increased deletion formation compared to the standard CRISPR/Cas system. We performed a case study for the use of the CRISPR-EXO system as a potential anti-cancer therapeutic tool. In the case of our new system, we showed significant increase in cell death due to higher genome modification in BCR-ABL1 region. Later, these findings were confirmed also in an animal cancer model, where animals with tumors, electroporated with CRISPR-EXO system showed full survival and drastic reduction in tumor size.

Conclusions CRISPR-EXO upgraded CRISPR system based on tethering Cas9 protein to exonuclease EXOIII by heterodimeric coiled-coil forming peptides, resulted in highly efficient editing of BCR-ABL1 fusion gene, leading to enhanced death of CML cancer cells.

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P09.07 AN IMMUNE MODULATORY VACCINE TARGETING CCL22 PROMOTES ANTI-TUMOR IMMUNITY

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Background CCL22 is a macrophage-derived chemokine that exerts immunosuppressive functions by the recruitment of regulatory T cells (Treg) through the CCL22/CCR4 axis. It has been described to play a key role in the suppression of anti-cancer immunity in different cancer types including ovarian, breast, or pancreatic cancer and is thought to promote the suppression of anti-cancer immunity by Treg recruitment. Recently, we described that CCL22-specific T cells generated from cancer patients can kill CCL22-expressing tumor cells and directly influence the level of CCL22 *in vitro*.¹ In this study, we provide PoC data for a CCL22-targeting vaccine by assessing the immunotherapeutic efficacy of this approach in syngeneic mouse tumor models.

Materials and Methods Peptide vaccines that induce expansion of CCL22-specific T cells were identified by measurement of vaccine-induced *ex vivo* response (IFN γ ELISpot) in BALB/c and C57BL/6 mice. The antitumor efficacy was evaluated in CT26, Pan02 and B16 syngeneic models. To investigate the vaccine's mode of action, the tumor immune infiltration was analyzed through flow cytometry and qPCR.

Results Vaccination with CCL22-specific peptide vaccines induced expansion of primarily CD8+, CCL22-specific T cell responses (assessed by *ex vivo* IFN γ ELISpot). Treatment with CCL22 vaccines reduced tumor growth and increased survival in CT26, Pan02 and B16 tumor models. Assessment of gene expression in the tumors indicated that vaccination leads to a reduction of CCL22 expression in the tumor microenvironment (TME), as well as the expression of other immune-suppressive molecules such as IDO. Furthermore, vaccinated mice harbored an increased CD8+ T cell infiltration with a concomitant increase in M1/M2 ratio within the TME.

Conclusions This study provides evidence that targeting CCL22 expressing cells by vaccination induces immune modulation in the TME, leading to augmentation of anti-tumor responses - thus provides a rationale for a novel immunotherapeutic approach in cancer.

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P09.08 CLINICAL-GRADE MANUFACTURING OF ROR1 CAR T CELLS USING A NOVEL VIRUS-FREE PROTOCOL

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Background Immunotherapy with T cells that were modified by gene-transfer to express a ROR1-specific chimeric antigen receptor (ROR1 CAR-T) has therapeutic potential in ROR1⁺ malignancies in hematology and oncology. The ROR1 tumor