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%. 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.

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, electropropared 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.

REFERENCES

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P09.07 AN IMMUNE MODULATORY VACCINE TARGETING CCL22 PROMOTES ANTI-TUMOR IMMUNITY
1I Lecoq*, 1K Lopp, 1R Christensen, 2E Martinenaite, 1AW Pedersen, 1,2MH Andersen.
1IO Biotech, København, Denmark; 2Centre for Cancer Immunotherapy, København, Denmark.

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. 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 (IFNγ 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 (assayed by ex vivo IFNγ 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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antigen has a favorable expression profile with absence in vital normal human tissues. In this study, we sought to establish and validate clinical-grade manufacturing of ROR1 CAR-T to enable a Phase I/IIa clinical trial. In particular, we sought to integrate virus-free gene-transfer based on Sleeping Beauty transposition into this manufacturing protocol to permit scale-up and export to point-of-care manufacturing, and to reduce turn-around time, complexity and regulatory burden associated with conventional viral gene-transfer (biosafety level 2 to biosafety level 1).

Materials and Methods Buffy coats or leukaphereses were obtained from healthy donors to perform protocol optimization (n=7) and scale-up runs (n=1). CD4+ and CD8+ T cells were isolated separately by magnetic selection and stimulated with CD3/CD28 TransACT® reagent. T cells were transfected with mRNA encoding hyperactive Sleeping Beauty transposase (SB100X) and minicircle DNA (MC) encoding a pT2 transposon comprising the ROR1 CAR and an EGFRt marker gene using the MaxCyte GTx® electroporation platform. Following transfection, T cells were expanded for 10–13 days in G-REX® bioreactors and then harvested and formulated into the drug product at a 1:1 ratio of CAR-expressing CD4+CD8 T cells. The drug product underwent comprehensive phenotypic, functional and genomic analyses as part of product qualification.

Results The set of protocol optimization runs resulted in a highly robust process. On average, the stable gene-transfer rate at the end of the manufacturing process was 71% in CD4+ (n=5) and 54% in CD8+ T cells (n=7). The average yield of ROR1 CAR-T relative to the number of input T cells was 12.6-fold for CD4+ and 9.4-fold for CD8+ after 12–15 days of expansion, with an average viability of 84% for CD4+ and 82% of CD8+ T cells. The scale-up run was performed with a leukapheresis product from which 52.5 × 10^6 CD4+ and 109 × 10^6 CD8+ T cells were transfected. At the end of the manufacturing process (day 12), there were 844 × 10^6 CAR-expressing CD4+ (~16-fold expansion) and 857 × 10^6 CAR-expressing CD8+ T cells (8-fold expansion). In functional testing, ROR1 CAR-T showed specific recognition and potent elimination of ROR1+ target cells, as well as antigen-dependent cytokine production and productive proliferation in in vitro analyses. Experiments to determine the anti-tumor potency of the drug product in vivo and detailed genomic analyses are ongoing. Preliminary analyses suggest a favorable genomic insertion profile of the CAR transposon, and a transposon copy number that is well within the range acceptable for clinical use of the drug product.

Conclusions With this novel protocol, we aim to obtain the first manufacturing license for CAR-T in Europe that integrates our optimized approach with SB100X mRNA and transposon MC for CAR gene-transfer on the MaxCyte transfection platform. The quality and yield of the drug product support the design and dose escalation of the proposed clinical trial with ROR1 CAR-T, and will serve as a blueprint for other CAR-T products from our pipeline.


Abstracts

Background Despite early surgical debridement and application of systemic antifungal drugs, invasive fungal infections by mucor spp. are still associated with a very poor prognosis in immunocompromised patients. Due to their lack of immune defense, targeted treatment strategies reversing the hyporesponsiveness of the immune system by immune checkpoints might improve patients’ outcome. Until today, a successful recovery of mucormycosis after receiving anti-PD-1 antibody is only described once for a polytrauma patient. Therefore, we here describe the first immunosuppressed patient treated with nivolumab for invasive mucormycosis with aspergillus coinfection.

Materials and Methods A 51-year-old woman from Germany with acute myeloid leukemia (AML) relapse after allogeneic hematopoietic stem cell transplantation was treated with azacitidine and lenalidomide. She acquired an invasive fungal infection with mucor species *Lachttlebia ramosa* combined with *Aspergillus fumigatus* in functional panctoypenia. Three surgical pan.mitavecisions were performed and high dose i.v. antifungal treatment with liposomal amphoterinin B and isavucanozole was initiated. Due to missing treatment response with daily mucor progression nivolumab 240 mg was administrated and complemented by interferon γ (100μg s.c. 5 doses). Administration was repeated every 2 weeks (in total 4 doses of nivolumab, but only 10 doses of interferon γ due to recurrent fever episodes) and simultaneously i.v. antifungal treatment was deescalated. Blood samples were collected before (baseline treatment (BT)) as well as 2 weeks (under treatment (UT) 1) and 5 weeks (UT2) after treatment initiation with nivolumab. Peripheral blood mononuclear cells were isolated and flow cytometry analyses of lymphocytic subsets were performed.

Results Ten days after first dose of nivolumab, long-term local hemostasis was achieved. Local symptoms disappeared, sinisits complaints improved, and infiammation values decreased significantly. Sixteen days after treatment initiation a CT scan revealed a partial remission of mucormycosis invasion. Follow-up CT scans showed a stable disease. Expression of PD-1 on T cells was monitored as proof of concept from BT on and showed a significant reduction from 34.7% to 3.3% (UT1) and 1.38% (UT2). Both activation markers CD86 and CD69 showed an increase from BT to UT1. T cells showed high maturation markers throughout monitoring, while B cell maturation increased from BT to UT1/2. Nine