Neoantigens that fail to elicit measurable T cell responses following peptide immunization can control tumor growth when delivered using a Listeria-based immunotherapy platform

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ABSTRACT

Introduction: Recent advances in the field of cancer immunotherapy have identified CD8+ T cell responses against tumor-specific neoantigens as a key driver of tumor regression and prolonged survival. ADXS-Neo is a personalized Listeria monocytogenes (LM)-based immunotherapy designed to generate immune responses against mutation-derived tumor-specific neoantigens. ADXS-Neo’s unique immunotherapeutic advantage is the high-affinity bacterial vector that is engineered to secrete a fusion protein consisting of a truncated non-mammalian cytotoxic T lymphocyte (CTLA-4) which has an Adjacent position, and tumor-specific neoantigens that harbors nonmycobacterial point mutations (DNMG). The objective of the in vitro study is to demonstrate the feasibility of using the ADXS-Neo platform to target tumor-specific neoantigens to generate neoantigen-specific T cells and control tumor growth.

Results: Whole-exome sequencing of the MC38 mouse tumor cell line identified 2870 unique neoantigens. Among these, the UCSC 138 neoantigens were predicted to be less than 300 bp by netMHCcons. We evaluated the neoantigenic stability of 37 neoantigens and found that 12 neoantigens elicited a CD8+ T cell response following peptide immunization. Moreover, we identified 10 additional neoantigenic hits in HLA-A*02:01-bearing mice with a check point inhibitor. Altogether, we identified 22 neoantigens and 23 neoantigenic peptides. Two alternative HLA-A*02:01 donors, Lm19 and Lm20, targeting nonimmunogenic and immunogenic neoantigens respectively. The ability of both Lm19 and Lm20 to control MC38 tumor growth was evaluated in C57BL/6 mice. We found that both Lm19 and Lm20 led to an accumulation of neoantigen-specific CD8+ T cells and significantly slowed tumor growth. However, both Lm19 and Lm20 decreased the frequency and absolute number of intratumoral Tregs, TAFs, and MDSCs and increased the frequency and absolute number of effector CD8+ T cells. Interestingly, expression of PD-L1 was decreased in TAFs and MDSCs and the frequency and total number of granzyme A+ CD8+ effector T cells was increased. Furthermore, the percentage of phenotypically exhausted PD-L1+CD8+ T cells was decreased. Together, these data suggest that the microenvironment in mice receiving Lm19 and Lm20 become more cytotoxic and less suppressive.

Conclusions: ADXS-Neo is a potent immunotherapy capable of driving immune responses against tumor-specific mutations and leading to tumor control. The effectiveness of the in vivo platform demonstrated through the generation of neoantigen-specific T cells to peptide sequences that were “non-immune” using a conventional peptide-adjuvant immunization. This study is a clear demonstration that 1 T cell-mediated anti-tumor responses can be generated by targeting neoantigen-derived peptides with the ADXS-Neo Listeria monocytogenes vectors.

OBJECTIVE

Evaluate the capacity of the “non-immune” Lm19 and “immunogenic” Lm20 ADXS-Neo constructs to generate neoantigen-specific T cells and control tumor growth in the MC38 mouse colorectal tumor model.

MATERIALS AND METHODS

Comparative Whole Exome Sequencing (WES): neoantigen prediction, and construct design: WES: MC38 mouse colorectal cell line and matched C57/6 normal tissue (tail snap) were sent for nucleic acid extraction, whole exome sequencing sample preparation, and comparative whole exome sequencing. Following WES, the whole exome sequence of the normal sample was used as a reference dataset to identify non-immunogenic amino acid changes present only in the MC38 tumor sample.

Neoantigen selection: Non-mutated neoantigens were run through netMHCCons (http://tools.immuneepitope.org/) and MDSCs with an IEDB score < 0.5 were selected for further experimentation. Peptides of the predicted 9-mer epitopes from 37 neoantigens identified from netMHCCons were used to immunize C57BL/6 mice with CpG adjuvant. Splenocytes were harvested and CD8+ T cell responses were evaluated using ELISPOT with 9-mer epitope re-stimulation. Additional neoantigens were identified in MC38 tumors following treatment with checkpoint inhibitors and ex vivo RNA. ELISPOT with epitope re-stimulation was performed.

Tumor model and in vitro: MC38 (300,000) cells were implanted subcutaneously (sc) in the right flank of mice and given 1 week of daily immunization with Lm19 starting on day 8.

Flow analysis: Tanser were enzymatically dissociated into single cell suspensions using a gentiMAXC and MACS tissue dissociation kit (Miltenyi Biotec). The resulting single-celled suspensions were immunopurified with the conjugated antibodies using standard staining procedures. Intracellular staining was performed as described above CD8+ T cells.

ELISPOT: Tansers were enzymatically dissociated into single cell suspensions using a gentiMAXC and MACS tissue dissociation kit (Miltenyi Biotec). Cells were isolated using pre-2 haloation kit. ELISPOT plates (Multibiotec) were prepared using standard methods, and 240 sample T cell were incubated with minimal Tiler epitopes corresponding to the predicted NCBI-9, or TIL binding peptide identified using netMHCCons.

RESULTS

Figure 1: Neoantigen Identification and Candidate Design

Figure 2: Tumor Microenvironment Dosing Schedule and Early T cell harvest following peptide immunization.

Figure 3: Intratumoral Regulatory T cells are Attenuated Following Treatment with ADXS-Neo

Figure 4: ADXS-Neo Therapy Attenuates the Suppressive Tumor Microenvironment

Figure 5: Influx of Cytotoxic TILs Following Treatment with ADXS-Neo

Figure 6: Frequency of Exhausted CD8+ TILs Decreases Following ADXS-Neo Treatment

Figure 7: Neoantigen-specific CD8+ TILs Following Immunization with ADXS-Neo

Figure 8: ADXS-Neo Controls Tumor Growth Targeting Either “Immune” or “Non-immune” NS5A

Figure 9: TCM8 tumor bearing mice immunized with Lm19 and rechallenged with minimal predicted epitopes previously used to identify neoantigens.

SUMMARY AND CONCLUSIONS

• ADXS-Neo controls tumor growth by targeting NS5A expressed as a dominant non-myobacterial vector.

• ADXS-Neo controls tumor growth and generates neoantigen-specific CD8+ T cells by targeting neoantigens that fail to elicit immunogenicity when immunized as peptide + CpG adjuvant.

• ADXS-Neo immunotherapy platform is a potent inhibitor of the suppressive TME by reducing numbers and suppressive phenotype of Tregs, MDSCs, and TAMs.

• ADXS-Neo generates a robust accumulation of cytotoxic and neoantigen-specific CD8+ T cells that control tumor growth.

REFERENCES