Viral based vaccine for personalized neoantigen-directed cancer therapy

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**BACKGROUND**

Anarchic cellular proliferation and deficient DNA repair mechanisms result in accumulation of mutations in cancer cells potentially leading to expression of tumor specific neoantigens (TSNA). Given their ad hoc onset in the tumor, TSNA are not subject to central tolerance and may constitute ideal targets for therapeutic cancer vaccines. However, clinical implementation of TSNA directed vaccination requires a potent immunizing vaccine formulation allowing the reproducible generation of a bespoke vaccine for every patient within acceptable time and cost. Herein, we propose a workflow to meet both requirements using a modified vaccinia Ankara (MVA) based vaccine referred to as MyVac™. Technical feasibility was shown, and immunogenicity of the vaccine was demonstrated using an exemplar patient with Non Small Cell Lung Cancer.

**METHOD**

Blood and tumor tissue sample from a patient with lung adenocarcinoma were sequenced and compared to reference genome to identify tumor specific variants. Consequently, identified mutations were assembled as a polyepitopic sequence in a recombinant MVA. The said viral vaccine was used to immunize human HLA-transgenic mice (HHD), and CD4 and CD8 cellular responses against the target neoantigens were assessed by ELISPOT. Response obtained in the HHD murine model were compared to spontaneous responses observed in the patient.

**IDENTIFICATION OF PATIENT SPECIFIC MUTATIONS**

Exome sequence was aligned to the reference genome using SpeedseqWBA; somatic variants were identified with Speedseq somatic and annotated with SnpEff. Variants were filtered (GSDS database) using the following filters: normal and tumour sequencing depth ≥ 10; genotype quality score of ≥ 320 (99% confidence), normal genotype is homogenous reference, tumour genotype is not homogenous reference. This yielded 2,218 variants that were further filtered using the following criteria: variant occurs in coding region, at least one observation of the variant in RNAseq with no observations in the normal transcriptome. Twenty-three variants were identified of which 22 were missense variants and one frame shift variant through deletion. The mutations were observed in 18 different genes.

**IMMUNOGENICITY OF VACCINE IN HHD TRANSGENIC MICE (HUMAN HLA-A2)**

HDD mice were immunized by I.V route at D1 and D7 with 10^7 pfu of MVATG19111 or MVATGN33.1. Immunogenicity was evaluated in splenocytes at D14, by ELISPOT using overlapping 20mer peptides. For highly immunogenic peptides, ELISPOT was repeated in presence of class I HLA or anti CD8 depleting beads. Additionally, we performed prediction of immunogenicity using BIMAS and SYPFPESII algorithms across all tested mutations, predicted immunogens are highlighted in red.

**IMMUNOGENICITY OF MUTATIONS IN PATIENT**

Pre-existing immunity to the non-synonymous mutations identified by NGS was studied by IFNγ ELISPOT in patient PBMCs. Positive responses are highlighted in yellow.

- Spontaneous preimmunity was observed in PBMC against: mutated POCl8, mutated KEAP1, mutated NFIL1, mutated MAFF, mutated KIAA0408.
- Responses were restricted to mutated proteins and no cross reaction was detected against non mutated protein.

**GENERATION OF PATIENT SPECIFIC VACCINE**

A viral based vaccine was generated to target 18 mutations identified in the patient.

- Eighteen mutated sequences coding identified above were selected and assembled into three fusion cassettes:
  - peptides spaced by 5-aa linkers (GSGSG, SGSGS, GSSGS or GTGSGS) in 3 expression cassettes containing a signal peptide
  - 3 mutated fragments encoded 29mer peptides, 1 was 20mer, 1 was 30 mer, 1 was 31mer and 1 was 76mer
  - Mutated fragment were spliced by a linker sequence
- The three expression cassettes were cloned into the Transgene MVA vector, each cassette containing a TAG sequence for control of transgene expression. This MVA vector is referred to as TG19111.
- The corresponding control vector, empty of the mutated fragment was also made and is referred to as MVATGN33.1.

**In vitro expression of neoepitope fusions**

CEF were infected at MOI 0.2 by either MVA (MVATGN33.1; negative control), MVATGN33.1. Western-blotted performed 24 hr later using anti-TAG antibodies

**GMP-manufacturing of customized MVA product**

- 1 batch per patient: around 150 clinical doses at 10^7 pfu/dose
- c<10 weeks process for the generation of clinical lots of viral vaccine
- Fully integrated GMP-compliant aseptic process
- In house GMP facility for modular manufacturing of multiple lots to be certified by Q4 2018

**CONCLUSIONS**

- A polyepitope vaccines based on the MVA platform is able to induce clinically relevant immune responses in HHD mice. After administration in HHD mice, both CD4 and CD8 T cells responses were detected. These responses could be predicted by an in silico approach, highlighting the need for a built-in antigen selection system in the process.
- An accelerated workflow for the production of MyVac product, and for its aseptic manufacturing was successfully developed.
- Clinical translation will be initiated in 2019

**Correlation of preimmunity in the patient and vaccine induced immunity in HHD mice.**

- Immunity against mutations in the MAFF and KIAA0408 genes was observed in both settings
- Mutations in POCl8 and PHE8 elicited responses after vaccination, in HHD were not detected in patient
- Responses to mutations in POCl8, KEAP1 and NFIL1 were detected in the patient but not after vaccination in the HHD model. This discordance may be due to difference in antigen processing between the two species.