Viral based vaccine for personalized neoantigen-directed cancer therapy

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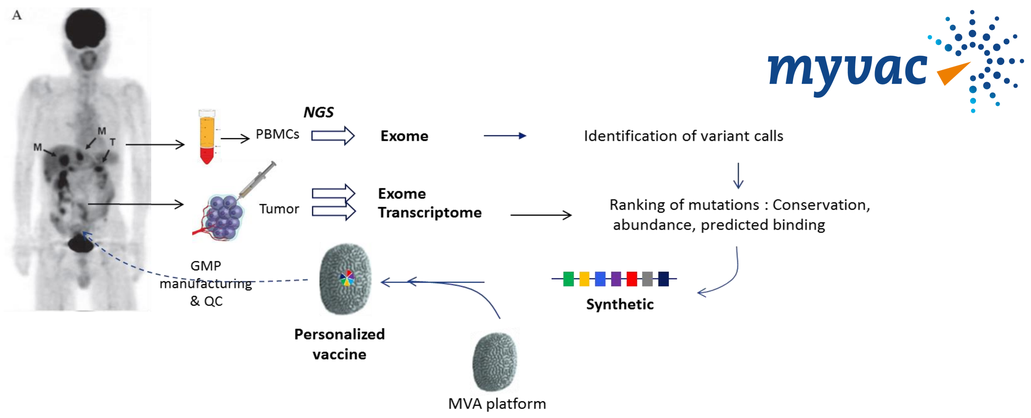
BACKGROUND

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 Speedseq/BWA; somatic variants were identified with SpeedSeq somatic and annotated with SnpEff. Variants were filtered (SQLite database) using the following filters: normal and tumour sequencing depth ≥ than 10, genotype quality score of ≥20 (99% confidence), normal genotype is homozygous reference, tumour genotype is not homozygous 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**.

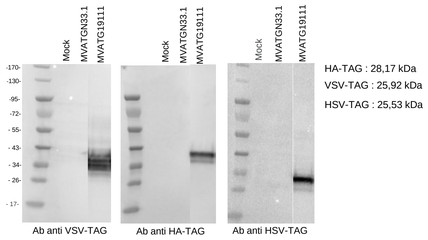
GENERATION OF PATIENT SPECIFIC VACCINE

A viral based vaccine was generated to targeted 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, GSTSG or SGTGS) 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 sperated 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

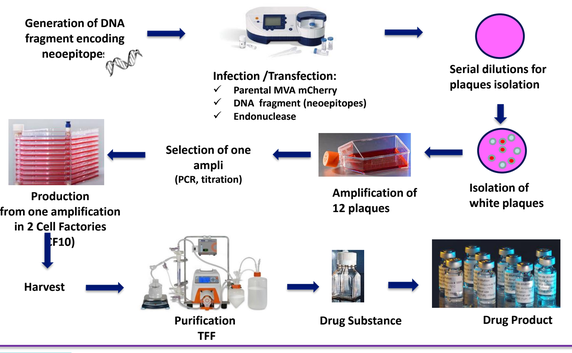
CEF were infected at MOI 0.2 by either MVA (MVATGN33.1: negative control), MVATG19111. Western-blot performed 24 h later using anti-TAG antibodies



HA-TAG : 28,17 kDa
VSV-TAG : 25,92 kDa
HSV-TAG : 25,53 kDa

GMP-manufacturing of customized MVA product

- 1 batch per patient: around 150 clinical doses at 10^8 pfu/dose
- <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



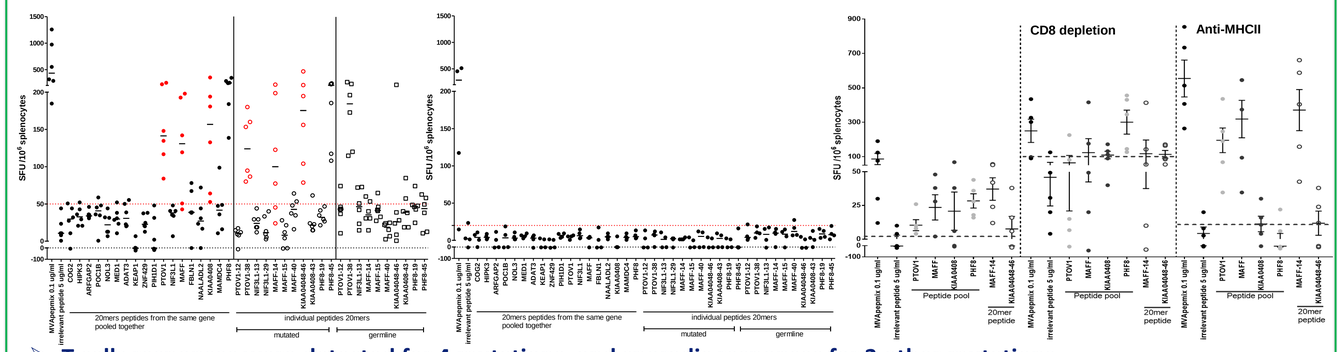
IMMUNOGENICITY OF VACCINE IN HHD TRANSGENIC MICE (HUMAN HLA-A2)

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 anti-class II MHC or of anti-CD8 depleting beads. Additionally, we performed prediction of immunogenicity using BIMAS and SYFPEITHI algorithms against all tested mutations, predicted immunogens are highlighted in red.

Responses against tumor specific antigens in vaccinated HHD mice

Responses in mice receiving an « empty » MVA vector

Responses against positive peptides after CD8 depletion or blocking of MHC II



- **T cells responses were detected for 4 mutations, and sporadic responses for 3 other mutations**
- **Responses were restricted to mutated proteins and no cross reaction was detected against non mutated protein**
- **Both CD4 and CD8 responses were detected in response to MVA vaccination**
- **3 out of 4 immunogenic peptides could have been predicted from sequencing data using in silico prediction**

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

Gene_Name	Peptide ID	Peptide Sequence
COG2	A003_m6_1	EIAGSSEAALTDVLEDAPAE
	A003_m15_26	PVYFQIRFREIAGSSEAALT
HIPK3	A003_m6_2	VKKLKAEPSSCVFQERNYPR
	A003_m15_27	QTQSSAFCSVKKLKAEPSSC
ARFGAP2	A003_m5_3	MAAERNKTEIQTLFKRLRAV
POC1B	A003_m6_4	FKPHAKAYRYVGHKDVVTSV
	A003_m10_28	MLWNFKPHAKAYRYVGHKDV
	A003_m15_29	WDTFLMLWNFKPHAKAYRYV
NOL3	A003_m6_5	NAQERLSETIDRERKRLVET
	A003_m15_30	GWDRAPTMGNAQERLSETID
	A003_m8_31	MGNAQERLSETIDRERKRLV
MED1	A003_m6_6	LPPEKQKHQTEDDFQRELFS
	A003_m15_32	KTKKKKSSRLPPEKQKHQTE
ADAT3	A003_m6_7	DGLPYVCTGYDLYVTREPCA
	A003_m15_33	AVRKLDADEDGLPYVCTGYD
KEAP1	A003_m6_8	VGVAVPWSPAGSRLTSRTVP
	A003_m15_34	TRMTSGRSGVGVAVPWSPAG
	A003_all_20	VPVEALLFLGQKYSPMGSI I
	A003_all_21	GQKYSPMGSI I VFVGKPGLK
	A003_all_22	EKTALQI THLPGREARMPQC
	A003_all_23	LKEKTALQI THLPGREARMP
	A003_all_24	I I VFVGKPGLKEKTALQI TH
	A003_all_25	AGSRLTSRTVPVEALLFLGQ
ZNF429	A003_m6_9	EMVDETPDGVSLLLPRLECS
	A003_m6_10	EMVDETPVVCSHFAEDFWPE
	A003_m15_35	KEPCKMKRHEMVDETPDGVS
	A003_m15_36	KEPCKMKRHEMVDETPVVCS
PIH1D1	A003_m6_11	NPEWRI MKNRPFMGSISQQN
	A003_m15_37	LEDKYNLQLNPEWRI MKNRP
PTOV1	A003_m6_12	PIGPSLPGLTLGGLAVSEHR
	A003_m15_38	GARVFGALGPIGPSLPGLTL
NIF3L1	A003_m6_13	ERLVILALENRVGIYSPHTA
	A003_m15_39	KRITWNTWKERLVILALENR
MAFF	A003_m6_14	ALMGLLVRELNRHLRGLSAE
	A003_m4_15	MGLLVRELNRHLRGLSAEEV
	A003_m15_40	ENTPHLSDEALMGLLVRELN
FBLN1	A003_m6_16	CEYSLI VGYQCGQVFQACCV
	A003_m15_41	RAAQAQGQSCEYSLI VGYQC
NAALADL2	A003_m6_17	QYLDNNDLQATALDLEWDME
	A003_m15_42	ADQRAPGHSQYLDNNDLQAT
KIAA0408	A003_m14_46	ALRRTTHNYTISLQSEALMV
	A003_m15_43	PALRRTTHNYTISLQSEALM
MAMDC4	A003_m6_18	GTTDFQSPEAGGWEDASVGR
	A003_m15_44	AGGEDEQACGTTDFQSPEAG
PHF8	A003_m6_19	CVGVEQEKAADIDLYHCPNC
	A003_m15_45	MCQDWFHGSCVGVEQEKAAD

- Spontenous preimmunity was observed in PBMC against;
  - mutated POC18
  - mutated KEAP1
  - mutated NIF3L1
  - mutated MAFF
  - mutated KIAA0408
- Responses were restricted to mutated proteins and no cross reaction was detected against non mutated protein

CORRELATION OF HUMAN AND MURINE RESPONSES

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

Mutated gene	Spontaneous responses in PBMCs	Responses in HHD mice after vaccination
MAFF	+	+
KIAA0408	+	+
PTOV1	-	+
PHF8	-	+
POC1B	+	-
KEAP1	+	-
NIF3L1	+	-

- Immunity against mutations in the MAFF and KIAA0408 genes was observed in both settings
- Mutations in PTOV1 and PHF8 elicited responses after vaccination, in HDD were not detected in patient
- Responses to mutations in POC1B, KEAP1 and NIF3L1 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.

CONCLUSIONS

- A polyepitope vaccines based on the MVA platform is **able to induce clinically relevant immune responses** in HHD mice**.** After administration in HDD 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 process** for the generation of MyVAC product, and for its aseptic manufacturing was successfully developed.
- **Clinical translation will be initiated in 2019**

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