DNA-encoded individualized neoantigen vaccines elicit durable and functional immune responses

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ABSTRACT

Individualized cancer vaccines based on tumor-specific neoantigens have garnered increasing attention after showing promising efficacy in recent clinical trials. Nykode Therapeutics developed a vaccine platform that targets antigens directly to antigen presenting cells (APCs). Individualized neoantigen vaccines delivered in a DNA-encoded format induce strong and broad T cell responses in pre-clinical and clinical settings.

Here we studied a DNA-encoded murine neoantigen vaccine that results in expression of a modular fusion protein containing a chemokine ligand 3-like 1 (CCL3L1) targeting domain to bind and attract APCs, a dimerization domain to facilitate strong bivalent binding, and an antigen unit encoding 20 MC38 (murine colon adenocarcinoma) neoantigens. The vaccine was administered by intramuscular injection followed by electroporation.

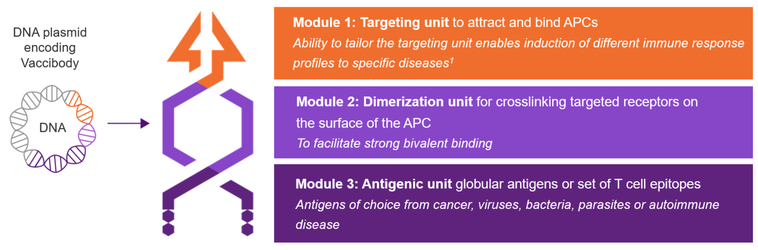
The durability of vaccine-induced immune responses was analyzed by measuring the number of persistent T cells and their ability to respond to boost vaccination. Animals were initially vaccinated with a prime (day 0) and boost (day 21) regimen and persistence was analyzed on day 133. Neoantigen-specific T cells persisted with only four- to five-fold reduced frequency on day 133 compared to day 28. To evaluate the ability of persistent T cells to respond to antigen re-exposure, animals were boosted by a 3rd vaccination on day 132. The majority of neoantigen-specific T cells were efficiently boosted (average 10-fold increase), resulting in a total frequency of neoantigen-specific IFN-γ+ T cells that was ~50% greater than the magnitude of responses observed on day 28. These data indicate a high number of persistent memory T cells that support robust responses to antigen re-exposure more than a hundred days following the initial prime-boost vaccination regimen.

The functional durability was assessed by tumor challenge either 90 days following single vaccination or 70 days following two vaccinations. A single vaccination was sufficient to reduce the frequency of tumor occurrence, while two vaccinations completely prevented tumor occurrence in all animals. These data indicate functional persistence of vaccine-induced neoantigen-specific T cells and highlight the importance of a prime-boost regimen in establishing long-lasting tumor protection.

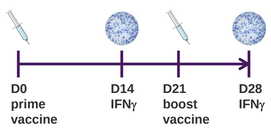
Analysis of patient samples from the N-01 phase 1/2a study (NCT03548467) of Nykode's individualized neoantigen vaccine in combination with checkpoint inhibitor treatment in patients with locally advanced or metastatic solid tumors demonstrates the translatability of these findings by showing that neoantigen-specific immune responses can be detected at least one year after the last vaccination.

Together, these data highlight the ability of Nykode's DNA-encoded neoantigen vaccine platform to induce functionally persistent T cell responses.

NYKODE'S APC-TARGETING VACCINE

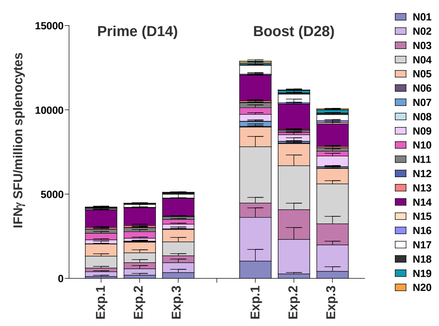


NYKODE'S NEOANTIGEN VACCINE ELICITS STRONG & BROAD CD8 T CELL RESPONSES

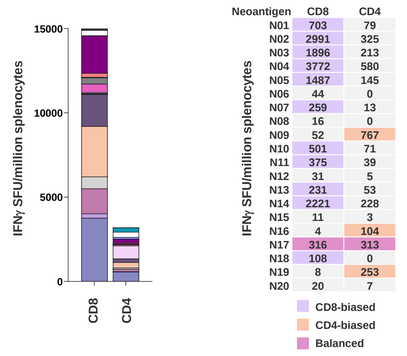


Experimental Design
- C57BL/6 mice (5/group)
- 20 µg DNA vaccine
- Intramuscular injection + electroporation
- IFNγ FluoroSpot (splenocytes)
- CD8/CD4 responses evaluated by T cell depletion

Strong & Broad Neoantigen Responses

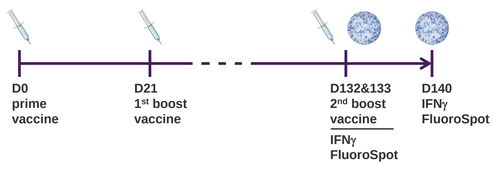


CD8-biased Neoantigen Responses



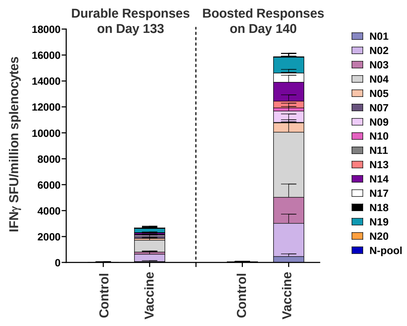
Neoantigen	CD8	CD4
N01	703	79
N02	2991	325
N03	1896	213
N04	3772	580
N05	1487	145
N06	44	0
N07	259	13
N08	16	0
N09	52	767
N10	501	71
N11	375	39
N12	31	5
N13	231	53
N14	2221	228
N15	11	3
N16	4	104
N17	316	313
N18	108	0
N19	8	253
N20	20	7

NYKODE'S NEOANTIGEN VACCINE ELICITS DURABLE T CELL RESPONSES



Experimental Design
- C57BL/6 mice (5/group)
- 20 µg DNA vaccine
- Intramuscular injection + electroporation
- IFNγ Fluorospot (splenocytes)
- Durable responses from first boost analyzed on day 133
- Ability to boost durable responses analyzed on day 140

Durable Neoantigen Responses



Durability of Individual Responses

Neo-Ag ID	Boosted response on D28	Durable response on D133	Fold reduction on D133
N01	588	91	6.5
N02	2069	568	3.6
N03	1289	165	7.8
N04	2774	912	3.0
N05	1128	140	8.1
N07	161	139	1.2
N09	479	106	4.5
N10	274	41	6.7
N11	206	1	386.0
N13	76	19	4.0
N14	1450	119	12.1
N17	463	13	34.7
N18	67	9	7.2
N19	143	317	0.5
N20	72	3	20.7
N-Pool	156	43	3.7

IFNγ SFU/million splenocytes

Median 6.6-fold reduction

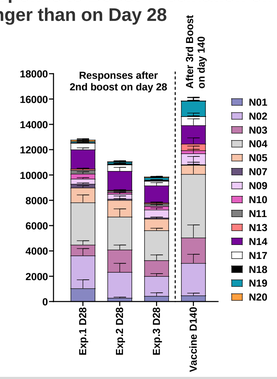
Boosting of Individual Responses

Neo-Ag ID	Durable response on D133	Boosted response on D140	Fold increased on D140
N01	91	478	5.3
N02	568	2557	4.5
N03	165	2012	12.2
N04	912	5026	5.5
N05	140	713	5.1
N07	139	42	0.3
N09	106	872	8.2
N10	41	242	5.9
N11	1	1	1.0
N13	19	523	27.2
N14	119	1460	12.2
N17	13	707	53.0
N18	9	8	0.9
N19	317	1211	3.8
N20	3	22	6.2
N-Pool	43	19	0.4

IFNγ SFU/million splenocytes

Median 5.4-fold increased
12/15 responses were boosted

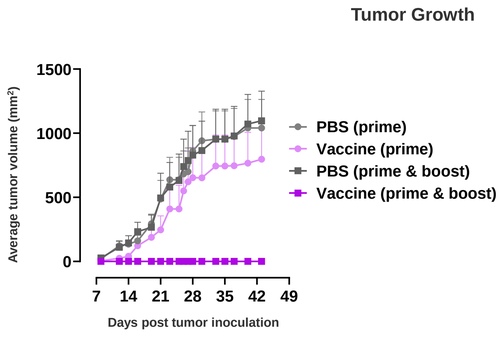
The Response to Boost Vaccination on Day 140 is Stronger than on Day 28

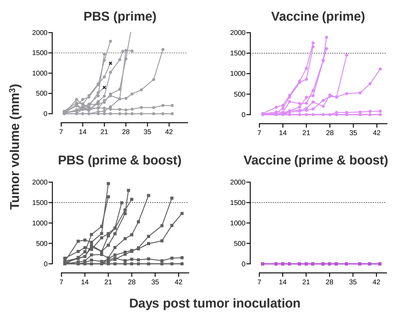


DURABLE TUMOR PROTECTION AFTER PRIME & BOOST VACCINATION

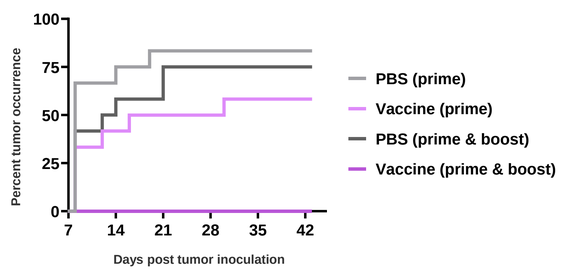
Experimental Design
- C57BL/6 mice (12/group)
- 80 µg DNA vaccine
- Intramuscular injection + electroporation
- Single (prime) or two (prime & boost) vaccinations
- 150 000 MC38 tumor cells injected subcutaneously on day 90
- Readout: tumor growth & survival

Tumor Growth





Tumor Occurrence



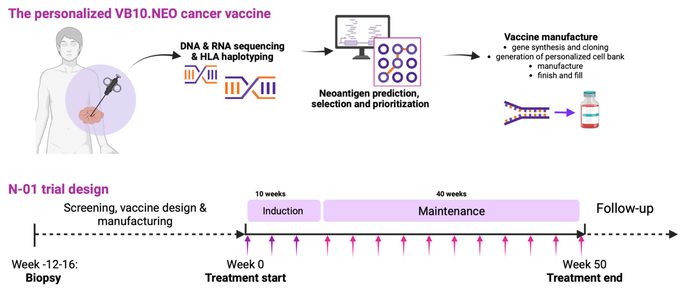
NYKODES'S VACCINE ELICITS DURABLE RESPONSES IN THE CLINIC

N-01 phase 1/2a study (NCT03548467)

Multicenter, open-label, first-in-human Phase 1b trial with repeated dose administration of VB10.NEO or VB10.NEO in combination with bempegaldesleukin (NKTR-214) immunotherapy in patients with locally advanced or metastatic solid tumors.

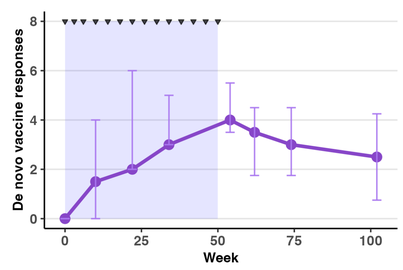
Neoantigens for inclusion in the VB10.NEO vaccine were selected by the NeoSELECT platform, which takes as input matched RNA-sequences, WES tumor/normal data, HLA-typing results and optional cfDNA WES data.

The individualized VB10.NEO vaccine was delivered by two intramuscular (IM) injections in the deltoid muscle, each of 0.5 ml for a total dose of 3 mg VB10.NEO using the CE-certified Stratis® Needle-free Injection System (PharmaJet®, Golden, Colorado, USA).



Abbreviations: CPI=checkpoint inhibitor; D=days; M=months; S=screening; SOC=standard of care.
Notes: All patients are on CPI at screening as the patient's SOC and according to currently approved indications.
Illustration created with BioRender.com.

Durability of De Novo Vaccine Responses



Duration of response was assessed in 8 patients by assaying the immunogenicity (ELISpot following in vitro stimulation for 12-14 days) of on treatment de novo responses in follow-up samples collected up to ~50 weeks post last dose of VB10.NEO. The latest positive follow-up timepoint defined the duration of response. IQR: Interquartile range.

SUMMARY

- **Nykode's neoantigen vaccine elicits strong & broad CD8-biased T cell responses in the murine MC38 tumor model.**
- **T cell responses persisted for more than 100 days and were efficiently boosted to levels that were comparable or stronger than was observed following the first boost vaccination on Day 28.**
- **Tumor growth was prevented by a prime & boost vaccine regimen, indicating that functional durability required boost vaccination.**
- **Nykode's personalized neoantigen vaccine VB10.NEO elicited durable responses in the clinic, highlighting the potential of our DNA-encoded vaccine platform to treat human cancer patients.**

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