Supplementary Materials for

Title: mRNA vaccine induced neoantigen-specific T-cell immunity in gastrointestinal cancer patients

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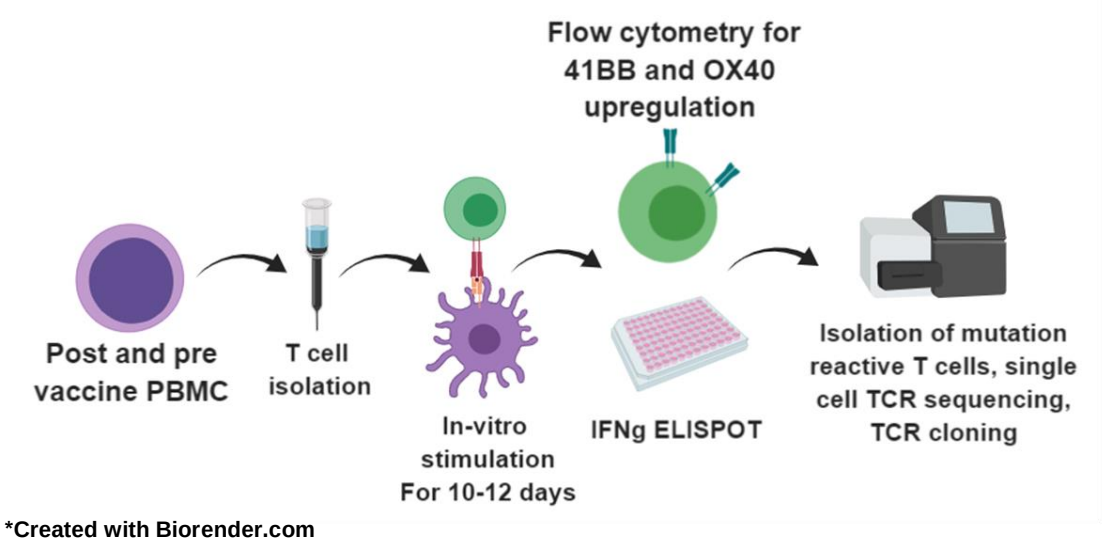
Supplementary figures: Figures. S1 to S2

Supplementary tables: Tables S1 to S5

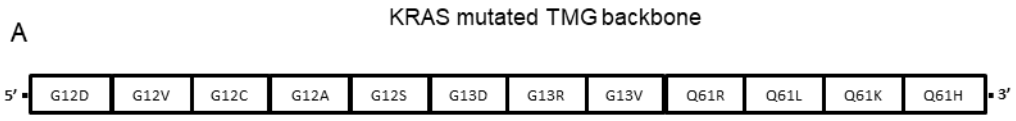
Supplementary figures



*Created with Biorender.com

Supplementary figure 1. Overview of the method used to asses immunogenicity of the mRNA vaccine.

A



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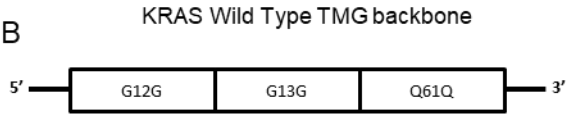

C

Long peptides (LP) and minigene 24 – 25 amino acid sequence

RAS site	mutation	Amino acid sequence	length
G12	G12 - WT	MTEYKLVVVGAGGVGKSALTIQLI	24
	G12D	MTEYKLVVVGADGVGKSALTIQLI	24
	G12V	MTEYKLVVVGAVGVGKSALTIQLI	24
	G12C	MTEYKLVVVGACGVGKSALTIQLI	24
	G12A	MTEYKLVVVGAAGVGKSALTIQLI	24
	G12S	MTEYKLVVVGASGVGKSALTIQLI	24
G13	G13 – WT	MTEYKLVVVGAGGVGKSALTIQLIQ	25
	G13D	MTEYKLVVVGAGDVGKSALTIQLIQ	25
	G13R	MTEYKLVVVGAGRVGKSALTIQLIQ	25
	G13V	MTEYKLVVVGAGVVGKSALTIQLIQ	25
Q61	Q61 – WT	ETCLLDILDTAGQEEYSAMRDQYMR	25
	Q61R	ETCLLDILDTAGREEYSAMRDQYMR	25
	Q61L	ETCLLDILDTAGLEEYSAMRDQYMR	25
	Q61K	ETCLLDILDTAGKEEYSAMRDQYMR	25
	Q61H	ETCLLDILDTAGHEEYSAMRDQYMR	25

Supplementary figure 2. Peptides and TMG used to evaluate T-cell reactivity to mutated KRAS. (A) Structure of the mutated KRAS TMG. (B) Structure of the WT KRAS TMG. (C) List of peptides used to evaluate T-cell reactivity to mutated KRAS.

Supplementary Tables

Patient	Course	Grade	Attributed to research	DLT	Serious	SAE	CTCAE	Adverse_Event
4251	1	1	No	N	No	N	5.0	Constipation
4251	1	1	Yes	N	No	N	5.0	Injection site reaction
4251	1	1	Yes	N	No	N	5.0	Injection site reaction
4251	1	1	Yes	N	No	N	5.0	Injection site reaction
4251	1	1	Yes	N	No	N	5.0	Injection site reaction
4251	1	3	No	N	No	N	5.0	Pain
4251	1	2	No	N	No	N	5.0	Shingles
4251	1	2	No	N	No	N	5.0	taneous tissue disorders -
4251	2	1	Yes	N	No	N	5.0	Injection site reaction
4271	1	1	No	N	No	N	5.0	Diarrhea
4271	1	2	Yes	N	No	N	5.0	Fatigue
4271	1	2	Yes	N	No	N	5.0	Flu like symptoms
4271	1	1	Yes	N	No	N	5.0	Injection site reaction
4271	1	2	Yes	N	No	N	5.0	Injection site reaction
4271	1	1	No	N	No	N	5.0	Pruritus
4271	2	1	No	N	No	N	5.0	Diarrhea
4271	2	2	Yes	N	No	N	5.0	Fatigue
4271	2	2	Yes	N	No	N	5.0	Flu like symptoms
4271	2	2	Yes	N	No	N	5.0	Injection site reaction
4289	1	2	No	N	No	N	5.0	Anemia
4289	1	3	No	N	No	N	5.0	Anemia
4289	1	1	Yes	N	No	N	5.0	Chills
4289	1	2	Yes	N	No	N	5.0	Fatigue
4289	1	2	Yes	N	No	N	5.0	Fatigue
4289	1	2	Yes	N	No	N	5.0	Fatigue
4289	1	1	Yes	N	No	N	5.0	Fever
4289	1	2	Yes	N	No	N	5.0	Flu like symptoms
4289	1	2	Yes	N	No	N	5.0	Injection site reaction
4289	1	2	Yes	N	No	N	5.0	Injection site reaction
4289	1	2	Yes	N	No	N	5.0	Injection site reaction
4303	1	2	Yes	N	No	N	5.0	Nausea
4303	1	2	Yes	N	No	N	5.0	Vomiting
4303	1	2	Yes	N	No	N	5.0	Fatigue
4303	1	2	Yes	N	No	N	5.0	Fever
4303	1	2	Yes	N	No	N	5.0	Injection site reaction
4303	1	2	Yes	N	No	N	5.0	Nausea
4303	1	2	Yes	N	No	N	5.0	Fatigue

Supplementary Table 1. Adverse events. DLT (Dose limiting toxicity), SAE (severe adverse events), CTCAE (common terminology criteria for adverse events)

Gene Name	transcript ID	AA change	Mut Epitope
TRAFD1	uc009zwb.2	p.R11L	MAEFLDDQETLLCDNCKKEIPVF
HNRNPU	uc001iba.1	p.F580I	AAQRRKMCLFAGIQRKAVVVCPKDE
RNF213	uc021uen.2	p.P4766H	HIVEQKNGKERVHILWHFLQKEAEL
FMOD	uc001gzr.3	p.S332N	LQGNRINEFSISNFCTVVDVVNFSK
METTL2B	uc011kop.2	p.R166W	LRDYGRYDMAQLWFKKGQCLSGNFY
LOC100288814	uc022bsu.1	p.R91C	DTFLPFEISMAQCFLLTASIFGFFG
FAM186B	uc010smk.2	p.C621Y	TMELGALRLQYLYHKYIFYRRLQSL
OR10H1	uc002nbq.2	p.E297V	FLSPIIFSLRNKVLKVAMKKTFFSK
ARMC9	uc031rrs.1	p.L302M	RPGTASTMLRASMAPVKLKDVPLLP
KCNB1	uc002xus.1	p.L333F	GFTLRRSYNELGFLILFLAMGIMIF
PRLR	uc021xxl.1	p.E116K	NATNQMGSSFSDKLYVDVTYIVQPD
NCAPD3	uc001qhd.1	p.L1349M	LQRLLPKARPMSMSTIAILNSVKKA
COL6A3	uc002vwr.3	p.G346W	EHVPQLLLLLTAWQSEDSYLQAANA
ITIH6	uc004dtj.2	p.Q816H	GLPQSRPGVSTLHVPKYPLHTRPRV
TCEB3	uc001bho.3	p.E196D	CHRMSPTYSSDPDSSDYGHVQSPPS
TMEM131	uc002syh.4	p.R1152S	YLEAQGIWEPFRSRLSFEASNPPFD
SPEN	uc010obp.1	p.T1260K	KVDEKVLPYSNIKVREESLKFNPYD
SLC12A6	uc010bau.3	p.S901N	ALLVAKNISFFPNNVEQFSEGNIDV
CRACR2A	uc010sen.1	p.P463L	EEPGTGEPGPGGLYPRPLRRIISVE

Supplementary Table 2. Vaccine sequences for patient 4251

Gene Name	transcript ID	AA change	Mut Epitope
TP53	uc031qyq.1	p.C96F	TCTYSPALNKMFFQLAKTCPVQLWV
DHTKD1	uc001ild.5	p.V643I	LEVSNSPLSEEAILGFEYGMSIESP
WDFY1	uc002vnq.3	p.E44K	LIPKEDGVITASKDRTIRVWLKRDS
USP47	uc001mjs.3	p.F1156L	REQCGLELSIDRLRLRKKTWKNPGT
CPSF6	uc001suu.4	p.G178E	QFEMQSRKTTQSEQMSGEGKAGPPG
CHD2	uc002bsp.3	p.K1351R	LKKRKPRVKKENRVPRLKEEHGIEL
BCLAF1	uc011ede.1	p.R143C	YRSSRSPRSSSSCSSSPYSKSPVSK
HPS3	ENST00000460822	p.L240H	DKNYTEDLSKLQHPLFRSWSHFQKT
NBPF8	uc021owe.1	p.S116L	YSTLSIPPEMLALYKSYSSTFHSLE
BTNL8	uc011dhh.2	p.R210H	LNGEHLYFTLNPHFISVFPRTPPTK
SYMPK	uc002pdq.2	p.A264T	NLTTALGSLANITRQRPMFMSEVIQ
IGF2BP3	uc003swg.3	p.T319M	DTKITISPLQELMLYNPERTITVKG
C1S	uc009zfr.3	p.P124S	QKKGWKLRYHGDSMPCPKEDTPNSV
NUDT5	uc001ilj.3	p.V144M	MDPGLSNCTIHIMTVTINGDDAENA
PCNXL2	uc001hvl.2	p.A1114V	CAVVAVLSFAVSVSTVFLSLRPFLS
PCGF3	uc011bva.1	p.G69S	IVIHQSHPLQYISHDRTMQDIVYKL
SYDE1	uc002naj.1	p.V196I	ATLTLLLDHLRLISSFHAYNRMTPQ
FAM172A	uc011cuf.2	p.N100K	KSFIFMSEDALTKPQKLMVLIHGSG
KRAS	uc001rgq.1	p.G12D	MTEYKLVVVGADGVGKSALTIQLI
HACE1	uc010kcy.1	p.N88I	QGVVREWFDILSIEIVNPDYALFTQ

Supplementary Table 3. Vaccine sequences for patient 4271

Gene Name	transcript ID	AA change	Mut Epitope
RAD21	uc003yod.3	p.D162V	GNISILQENDFGVFGMDDREIMREG
APC	uc011cvt.2	p.A1474fs	QVLPDADTLLHFARKVLQMDFLVHPA
PIK3CA	uc003fjk.3	p.E542K	QLKAISTRDPLSKITEQEKDFLWSH
KRT37	uc002hwp.1	p.R114H	GHEKETMKFLNDHLANYLEKVRQLE
NRROS	uc003fwv.3	p.T254M	LATGGEAAFELEMLDLSHNQLLFFP
NUDT6	uc003iex.3	p.K30E	TAVREVFEETGIESEFRSVLSIRQQ
OR8I2	uc010rix.2	p.F28L	GFANHPELQVSLLLMFLFIYLFTVL
SPATA31D1	uc004amn.3	p.G1456fs	NPEVHVRAEPVQGLSLQLQGSLLQSDTYQILQPTSYLCWPELSYKD
INHBC	uc001snv.1	p.R40Q	ACGGPTLELESQQELLLDLAKRSIL
CPAMD8	uc002nfb.3	p.V922I	CVAPGEAEPIWVILSFSDLGLNNIT
PIK3R5	uc021tqc.1	p.T63M	RRDSRSLEGSSDMALPLRRAGSLCS
TASP1	uc002woi.3	p.M323L	QAEDAHQALLETLQNKFISSPFLAS
OR52N2	uc010qzp.2	p.V19I	SSLTPGFFILNGIPGLEATHIWISL
OR52D1	uc010qzg.2	p.A281V	HHEVPKHVHIFLVNLYVLVPPVLNP
TRIM58	uc001ido.3	p.R181H	VIWKEKVEMQRQHFRLEFEKHRGFL
FTCD	uc010gqg.1	p.A316E	RRTAALQEGLRREVSVPLTLAETVA
SYNE1	uc003qow.3	p.K1007R	QASSRKCEEGKNRMLFVTVTLFKII
ZBTB21	uc021wjo.1	p.V749I	QGSHERLCRNAAICPYCSLRFFSPE

Supplementary Table 4. Vaccine sequences for patient 4289

Gene Name	transcript ID	AA change	Mut Epitope
TP53	uc010cnk.2	p.M55fs	ENNVLSPLPSQARMI
RBM42	uc002oaq.3	p.R294W	PLRIPELLSLRPWPRPPRPEPPPGL
KRAS	uc001rgq.1	p.G12V	MTEYKLVVVGAVGVGKSALTIQLI
DBN1	uc011dga.1	p.P369T	PPEIDITCWDADTVPEEEEGFEGGD
SLC12A2	uc010jdg.3	p.D202Y	GGGSGHHQHYYYYTHTNTYYLRTFG
PHF20L1	uc031tck.1	p.V298fs	QRLATLPMPDDSVRKGFFSLSSH
PPP2R4	uc011mbq.1	p.K141R	VLRVDDQIAIVFRVFNRYLEVMRKL
HPS1	ENST00000359632	p.D82Y	QDLYPSESTAEDYIQETDSFSLPEE
SEC23IP	uc010qtc.2	p.Q44H	QQVPARPGAPSVHVPSPFLLQNQYE
ZFYVE26	uc001xkc.4	p.S2211P	HGHSSKRQCLPYPSGLDHSALSGTI
RFX3	uc003zht.1	p.D393Y	QTFWRYSPSTPTYGTTITESRSEST
KMT2C	uc003wla.3	p.Y4586fs	ALFPVGYEASRLCTGALAMPIGAAATCAPLRRRMGAQCLSSGLWNKAMKTWF
MYOF	uc001kio.3	p.K1774T	GPPFNITPRKAKTYYLRVIIWNTKD
EIF4A2	uc003fqw.3	p.S229R	RDVIMREFRSGSRRVLITTDLLARG
WHSC1L1	uc011lbm.2	p.L1246F	LFALCDIPAGMEFTFNYNLDCLGNG
PCNXL2	uc001hvl.2	p.A199V	STSPGIKVESLPVSQAHMLETTTKS
PTK2	uc011ljr.2	p.L1047V	TAAHALAVDAKNVLDVIDQARLKML
ARHGEF1	uc002osc.3	p.R93Q	ESLRVSDRRRPSQGSLGAKGRGGGR
SETD4	uc021wiy.1	p.M63L	APACFPGTGRGLLSQTSLQEGQMII

Supplementary Table 5. Vaccine sequences for patient 4303

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	X	
		• Structured abstract recommended	X	
		• Information on target population or study sample	X	
Introduction				
Background	2	• Scientific background and explanation of rationale	X	
		• Theories used in designing behavioral interventions		X
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	X	
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	X	
		• Recruitment setting	x	
		• Settings and locations where the data were collected	x	
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	X	
		○ Content: what was given?	x	
		○ Delivery method: how was the content given?	x	
		○ Unit of delivery: how were the subjects grouped during delivery?	x	
		○ Deliverer: who delivered the intervention?	x	
		○ Setting: where was the intervention delivered?	x	
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	X	
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	X	
		○ Activities to increase compliance or adherence (e.g., incentives)	x	
Objectives	5	• Specific objectives and hypotheses	x	
Outcomes	6	• Clearly defined primary and secondary outcome measures	x	
		• Methods used to collect data and any methods used to enhance the quality of measurements	X	
		• Information on validated instruments such as psychometric and biometric properties		X
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	X	
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	X	
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	X	
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	X	

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	x	
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	x	
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	x	
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data		x
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis		x
		• Methods for imputing missing data, if used		x
		• Statistical software or programs used	x	
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	x	
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	x	
		○ Assignment: the numbers of participants assigned to a study condition	x	
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	x	
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	x	
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	x	
		• Description of protocol deviations from study as planned, along with reasons	x	
Recruitment	13	• Dates defining the periods of recruitment and follow-up	x	
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	x	
		• Baseline characteristics for each study condition relevant to specific disease prevention research	x	
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	x	
		• Comparison between study population at baseline and target population of interest		x
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences		x

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	x	
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	x	
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	x	
		• Inclusion of null and negative findings	x	
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	x	
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	x	
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	x	
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	x	
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	x	
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	x	
		• Discussion of research, programmatic, or policy implications	x	
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	x	
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	x	

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/