Comprehensive ctDNA monitoring provides early signal of clinical benefit with a novel personalized neoantigen directed immunotherapy for late-stage cancer patients

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gritstone bio

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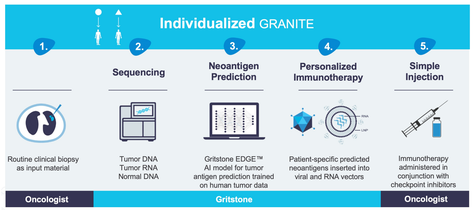
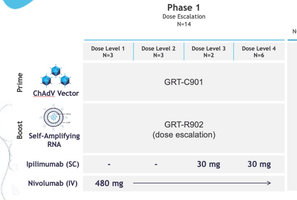
Abstract: Neoantigen directed immunotherapy holds promise for patients with solid tumors devoid of immune infiltration to benefit from immune checkpoint immunotherapy (CPI). A heterologous prime-boost vaccination approach consisting of a Chimpanzee Adenovirus (ChAd) prime and multiple self-amplifying mRNA (samRNA) boosts, delivering 20 neoantigens, has been evaluated in a Phase 1/2 clinical trial in late-stage solid tumor patients in combination with nivolumab and ipilimumab (NCT03639714). Neoantigen dynamics, tumor burden and genomic correlates of response were studied over time in 25 patients (12 GEA, 2 NSCLC, 11 MSS-CRC).

GRANITE process workflow



Methods: Exome sequences from archival (sample used for neoantigen selection), baseline (start of immunization) and on-treatment biopsies were analyzed for 25, 16 and 10 patients, respectively. Paired pre- and post-vaccine tumor transcriptomes were analyzed for 6 patients. Personalized capture baits were designed for all non-synonymous mutations detected in archival biopsies (mean 144; range: 67-402) for ctDNA monitoring. Longitudinal ctDNA samples were collected monthly on treatment (mean 7; range: 1-18). ctDNA duplex UMI libraries were captured and sequenced to a target mean raw depth >80,000x and reduced to 3x per strand consensus duplex reads.

Phase 1/2 clinical trial and demographics



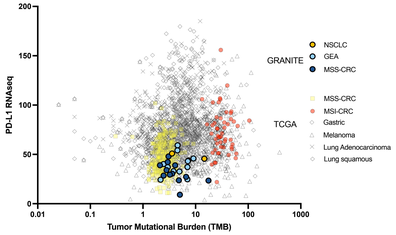
Demographics and Tumor Types	n=26
Age: mean (range)	60 (38-77)
Gender: Female/Male	9/17
Tumor Types	
Microsatellite-stable (MSS) Colorectal Cancer	11
Median number of prior therapies (range)	2 (2-3)
Gastroesophageal adenocarcinoma (GEA)	12
Median number of prior therapies (range)	1 (1-2)
Non-small cell lung cancer	2
Median number of prior therapies (range)	2 (2)
Prior anti-PD(L)1 therapy	2

SC = subcutaneous
GRT-C901 = chimpanzee adenovirus encoding 20 neoantigens
GRT-R902 = self-amplifying mRNA in lipid nanoparticles encoding same neoantigens as GRT-C901

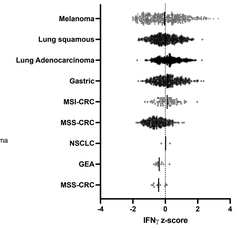
N=26 is the number of patients enrolled – G26 has not been analyzed yet.

Patient tumors – prior to GRANITE – are immune deserts

PD-L1 mRNA expression *vs.* Tumor Mutational Burden



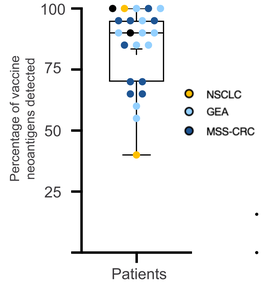
Interferon Gamma (IFN-γ) mRNA expression signature



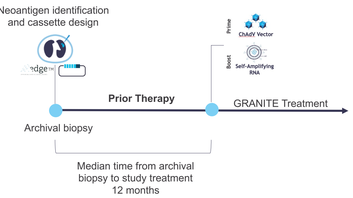
TMB calculated based on all somatic mutations called divided by WES bait set size
Average IFN-γ score utilizing established gene set across GRANITE and relevant PANCAN TCGA tissue types (LUAD, LUSC, COAD, SKCM): J Clin Invest. 2017;127(8):2930-2940

Most vaccine neoantigens are retained through prior treatments including chemotherapy

Vaccine neoantigen retention in treatment biopsies or ctDNA



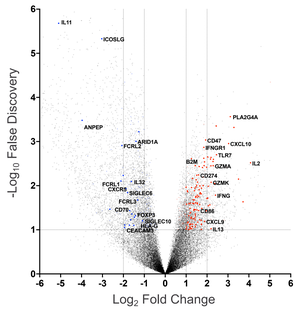
Individualized immunization requires tumor-informed neoantigen selection and manufacturing



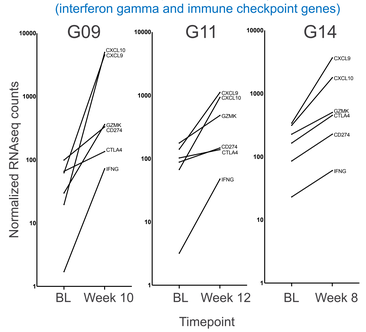
- Most of the vaccine neoantigens (average 83%; range 45%-100%) were detected in ctDNA and baseline or on-treatment biopsies.
- Most vaccine neoantigens are truncal – the majority of undetected neoantigens are subclonal.

Increase in interferon gamma and immune infiltration related genes in GRANITE biopsies post vaccination

Differential Gene Expression Analysis (pre- vs. post- GRANITE biopsies)



MSS-CRC Paired pre and post biopsy expression (interferon gamma and immune checkpoint genes)

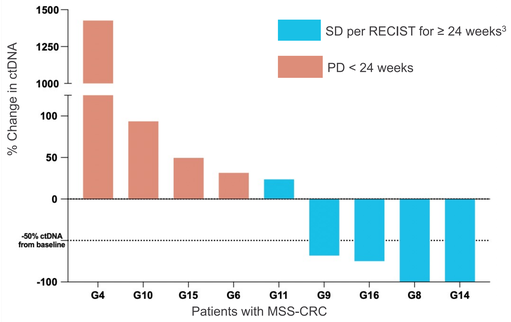


Differential gene expression analysis from 6 paired pre- and post-vaccine biopsies (3 GEA and 3 MSS-CRC – G09 with molecular response (MR*)) demonstrated significant upregulation in gene signatures associated with immune-inflamed tumor microenvironments including interferon gamma responses and immune checkpoint genes

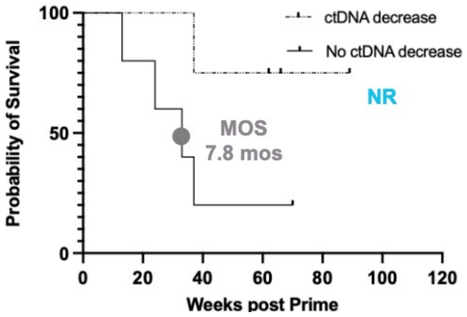
Pre-treatment biopsies include manufacturing (MFG) and baseline (BL) when available.
On-Treatment (OT) - G01 post-vaccine collected at week 80 (EOT) all others collected at weeks 8-14 post ChAd prime
*MR = > -50% decrease in ctDNA from baseline

ctDNA molecular response is associated with OS in patients with advanced stage MSS-CRC

Best molecular response (% ctDNA change from baseline)[1,2]



Overall Survival (OS)



1. ctDNA assessment based on Gritstone-developed, tumor-informed assay
2. ctDNA assessment not available for the 3 most recently treated patients
3. Patients G14 and G16 with PD at week 16 and 9 respectively not confirmed on subsequent scans through week 24

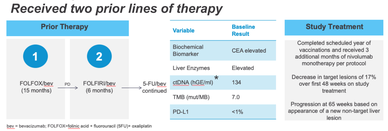
Data cut-off 05 Jan 2022

SD=stable disease; PD=progressive disease

Acquired resistance in antigen presentation pathway at 12 months of therapy suggesting vaccine induced immune pressure on tumor

Molecular response achieved for >7 months before subclonal TAP1 biallelic loss-of-function mutations are identified in ctDNA

G08: 50-year-old Female with Metastatic MSS-CRC
Received two prior lines of therapy



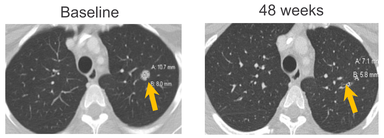
Variable	Baseline Result
Biochemical Biomarker	CEA elevated
Liver Enzymes	Elevated
ctDNA (hGE/ml) *	134
TMB (mut/MB)	7.0
PD-L1	<1%

Study Treatment

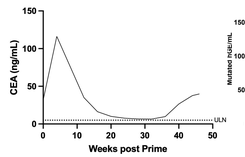
Completed scheduled year of vaccinations and received 3 additional months of nivolumab monotherapy per protocol

Decrease in target lesions of 17% over first 48 weeks on study treatment

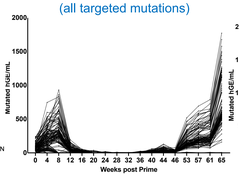
Progression at 65 weeks based on appearance of a new non-target liver lesion



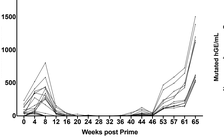
Longitudinal CEA dynamics



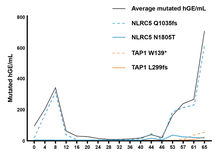
Longitudinal ctDNA dynamics (all targeted mutations)



Longitudinal ctDNA dynamics (Vaccine neoantigens)



Longitudinal ctDNA dynamics (subclonal escape mutations)



G08 ctDNA dynamics in panel 2 and 3 as an example of the correlation of vaccine targeted neoantigens and all monitored mutations. Across the GRANITE patient cohort mean neoantigen variant allele frequency (VAF) strongly correlated with all monitored mutations VAF (R^2 = 0.90, $p < 0.0001$) through treatment.

*hGE/ml=Mutated haploid genome equivalents, calculated from cfDNA concentration (ng/ml) and VAF.

Conclusion

- Majority of neoantigens are retained in tumor while vaccine is being manufactured
- Comprehensive ctDNA longitudinal monitoring enables real-time assessment of response/resistance and potentially provides more useful predictive information than traditional RECIST radiologic assessment in the context of this novel immunotherapy
- Neoantigen-directed Immunotherapy appears to drive clinical benefit in patients with advanced MSS-CRC, with molecular response elicited in 4/9 treated subjects and correlated with prolonged progression-free survival and overall survival