Class II-restricted CD4⁺ T cell responses to KRAS G12C in healthy donors linked to bacterial mimotope: Lessons for KRAS neoantigen vaccines in cancer patients

gritstone bio

Abstract 2267

Christine D Palmer[1], Meghan G Hart[1], Sonia Kounlavouth[1], Harshni Venkatraman[1], Lauren D Kraemer[1], Martina Marrali[1], Calixto Dominguez[1], Fatema Z Chowdhury[1], Jason R Jaroslavsky[1], Lindsey Arcebuche[1], Lorenzo Hernandez[1], Bukola Adeoye[1], Severino Cuison[1], Amy R Rappaport[1], Greg Boucher[1], Monica Lane[1], Melissa Rotunno[1], Kenneth Avocetien[1], Leiliane Sousa[1], Chris Puccia[1], Molly Likes[1], Rahul Vegesna[1], Rita Zhou[1], Alexis Mantilla[1], Matthew Davis[1], Ankur Dhanik[1], Melissa Johnson[2], Andrew R Ferguson[1], Karin Jooss[1]

[1]*Gritstone bio, Inc.,* [2]*Sarah Cannon Research Institute*

BACKGROUND

Mutations in the KRAS gene are among the most common driver mutations in cancer. Targeting KRAS G12C mutations has led to drug approvals, though acquired resistance mutations pose a challenge for small molecule-based monotherapies. Vaccines targeting KRAS mutations may enable durable tumor control and clinical benefit to patients with solid tumors. While cytotoxic CD8⁺ T cells are critical to tumor control and clearance, CD4⁺ T cell activation is a key component of a durable anti-tumor response. Cancer neoepitopes with homology to infectious disease antigens (mimotopes) were associated with long-term clinical benefit following CTLA-4 blockade. We identified several healthy donors with mimotope responses to KRAS G12C and assessed functionality of CD4⁺ and CD8⁺ T cell responses before confirming results in samples from a KRAS G12C patient.

METHODS

- Whole PBMCs or CD4⁺/CD8⁺-depleted PBMCs from healthy donors were screened via *ex vivo* interferon-gamma (IFNγ) ELISpot using KRAS G12C peptides and a bacterial lipoprotein peptide sequence with homology to KRAS G12C to identify donors with cross-reactive T cells. Polyfunctionality was assessed via intracellular cytokine staining (ICS).
- Cytotoxic capability of CD4⁺ T cells was evaluated by culturing class II expressing target cells presenting various G12C peptides (given arbitrary numbers; Peptide 11, 91, etc.) with PBMCs expanded by *in vitro* stimulation (IVS) and imaging using Incucyte® Live-Cell Analysis System.
- Vaccine-induced KRAS G12C-specific T cell responses from a patient with KRAS G12C-positive non-small cell lung cancer (NSCLC) receiving a shared neoantigen-targeting cancer vaccine consisting of Chimpanzee Adenovirus (ChAd) prime and self-amplifying mRNA (samRNA) boost vaccinations in combination with nivolumab 480 mg IV (NCT03953235) were assessed.
- Patient PBMCs were analyzed by *ex vivo* IFNγ ELISpot, and T cell receptor (TCR) and transcriptome single cell sequencing analyses were performed to identify antigen-specific TCRs.

KRAS G12C-specific T cell responses in healthy donors are driven by polyfunctional CD4⁺ T cells

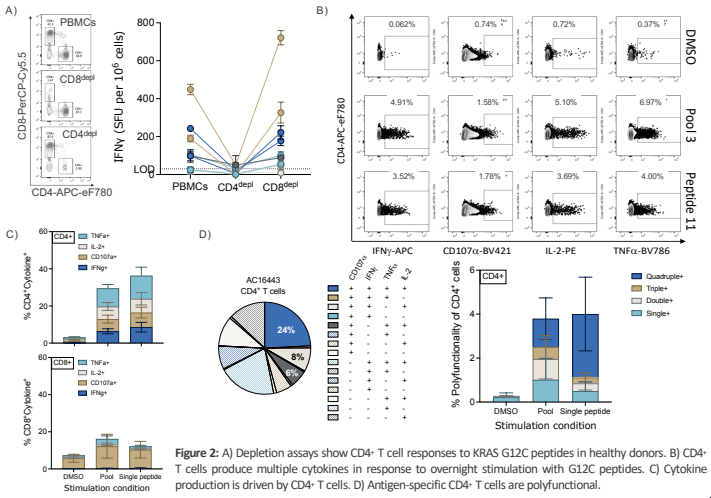

Figure 2: A) Depletion assays show CD4⁺ T cell responses to KRAS G12C peptides in healthy donors. B) CD4⁺ T cells produce multiple cytokines in response to overnight stimulation with G12C peptides. C) Cytokine production is driven by CD4⁺ T cells. D) Antigen-specific CD4⁺ T cells are polyfunctional.

Patient TCRs identified in post-vaccination sample are from CD4⁺ T cells with cytotoxic gene expression profile

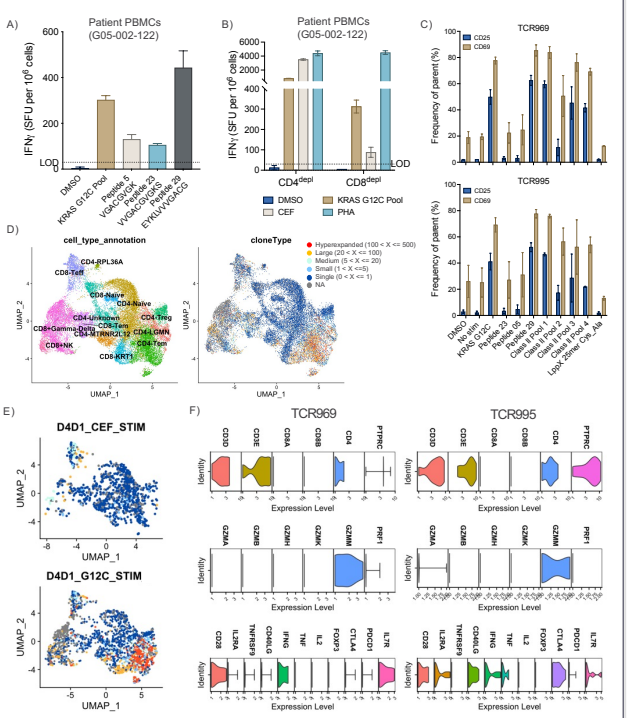

Figure 4: A) PBMC responses to G12C peptide pool and single peptides (# 5, 23 & 29) from vaccinated patient with KRAS G12C mutation. B) Mixed CD4⁺/CD8⁺ T cell response observed by *ex vivo* ELISpot C) TCRs identified from patient sample are functional when cultured with class II matched APCs presenting G12C peptides. D) Phenotypic clustering of patient samples. E) Expansion of clonotypes in patient sample stimulated with G12C is primarily from CD4⁺ T cell subsets (CD4⁺ Tem). F) G12C-specific patient TCRs are CD4⁺ T cell derived and have a gene profile indicating cytotoxic capability.

KRAS G12C T cell responses in healthy donors are associated with bacterial mimotope responses

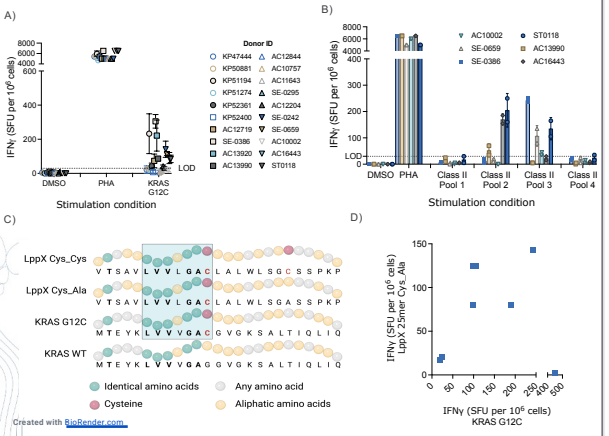

Figure 1: A) Healthy donor screening results in responses to G12C minimal epitope peptides. B) Select healthy donors show responses to 11-25aa G12C class II peptides. C) KRAS G12C sequence has homology to LppX lipoprotein found in *M. tb* and *E.coli*. D) G12C and LppX mimotope responses correlate in the majority of donors tested.

CD4⁺ T cell-dependent killing of target cells presenting KRAS G12C epitope in healthy donors

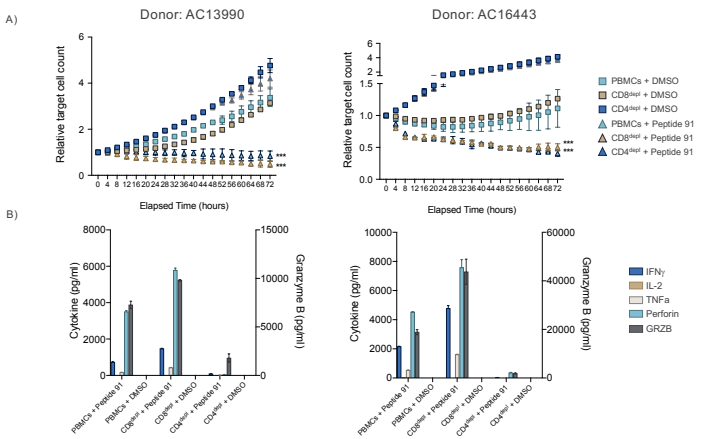

Figure 3: A) CD4⁺ T cell-mediated killing of target cells presenting G12C single peptide #91. B) Production of IFNγ, IL-2, TNFa, Perforin, and Granzyme B observed in total PBMC and CD8-depleted populations in supernatants collected from Incucyte® co-culture assay.

SUMMARY

- Healthy donor responses to KRAS G12C peptides are driven by CD4⁺ T cells which likely have cross-reactive TCRs that also recognize a bacterial mimotope.
- G12C-specific CD4⁺ T cells in healthy donors are polyfunctional and cytotoxic.
- KRAS G12C-specific CD4⁺ T cells were identified from a patient with KRAS G12C-positive NSCLC receiving a shared neoantigen-targeting cancer vaccine + Nivolumab (NCT03953235).
- Patient had progressed on prior ICB therapy, but had positive clinical outcome after vaccination, indicated by tumor shrinkage and reduction in ctDNA levels
- TCR analysis in a patient sample indicates expansion of CD4⁺ T cell clones after G12C peptide stimulation. TCRs identified are functional *in vitro* and cells expressing these TCRs upregulate genes associated with cytotoxic CD4⁺ T cell function.
- Healthy donors with mimotope responses can be used in proof-of-concept research to investigate KRAS G12C neoepitopes that may inform future therapeutic approaches.