A novel microbiome-derived peptide, SG-3-00802 reverses resistance to anti-programmed death protein-1 (PD-1) therapy

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SECOND GENOME

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ABSTRACT

Background

Despite the clinical success of immune checkpoint inhibitors (ICI) in many cancers, a large unmet need exists, as many patients respond inadequately. Fecal microbiome transplant from anti-PD-1 responders into non-responders improves response to ICI, providing a strong rationale that gut microbiome influences response to ICI. Using Second Genome's proprietary algorithms, we derived an ICI responder-specific microbial signature. Here, we report the antitumor activity and immunomodulatory functions of a novel, microbiome-secreted peptide SG-3-00802.

Results

SG-3-00802 showed robust CXCL10 induction *in vitro* and markedly increased CXCL10 and CD8 levels in tumors, without affecting IFN-γ and IL-12. SG-3-00802 showed improved antitumor responses in combination with anti-PD-1 in the anti-PD-1-insensitive RENCA mouse model vs control peptide or anti-PD-1 antibody alone. Treatment with SG-3-00802 ± anti-PD-1, significantly improved overall survival with many mice showing complete tumor regression. Surviving mice with fully regressed tumors (83%) rejected newly implanted tumors when rechallenged with RENCA cells, showing that the SG-3-00802 + anti-PD-1 combination yields long-lasting anti-tumor memory responses.

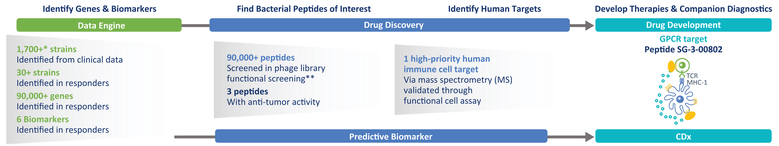
Using an LC MS/MS-based platform, we identified CXCR3, CXCR4, and CCR7 as putative binding partners of SG-3-00802. G-protein-coupled receptor activation assays in reporter cells revealed that addition of SG-3-00802 enhanced the activity of CXCR3 in the presence of its innate ligand CXCL11, validating CXCR3 as the functional target of SG-3-00802. This observation was confirmed *in vivo*, as CXCR3 inhibition decreased the activity of SG-3-00802 alone and in combination with anti-PD-1, supporting our hypothesis that *in vivo* activity of SG-3-00802 is CXCR3 dependent.

Conclusions

In summary, we showed that the microbiome directly interacts with the human immune system to impact antitumor immunity. SG-3-00802 shows great potential to improve responses in ICI-resistant patients and potentially acts as a CXCR3 receptor sensitizer by increasing CXCR3 activation in response to its ligands CXCL9/10/11, leading to recruitment of effector cells to mount a robust immune response.

BACKGROUND

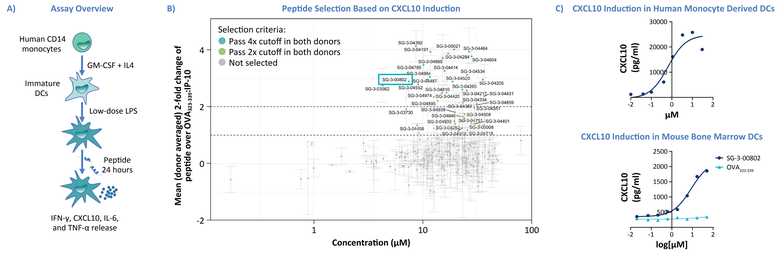
FIGURE 1:
Second Genome's Drug Discovery Platform



GPCR, G-protein coupled receptor; CDx, companion diagnostics; MHC-1, major histocompatibility complex class 1; TCR, T-cell receptor.
***Source Data:** Matson (Science 2018),[1] Gopalakrishnan (Science 2017),[2] Frankel (Neoplasia 2017)[3]; results from published data sets are confirmed in a proprietary SG KnowledgeBase.
**Screening using T cells, PBMC, monocytes, and dendritic cells.

FINDINGS

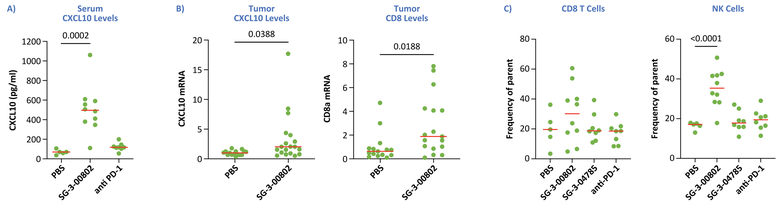
FIGURE 2:
SG-3-00802 induces CXCL10 secretion by human and mouse dendritic cells



CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; DCs, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, inducible protein 10; LPS, lipopolysaccharide; ova, ovalbumin; PBS, phosphate buffered saline; TNF, tumor necrosis factor.

Figure 2. (A) Schematic representation of the strategy used to evaluate the effects of microbiome-derived peptides in DCs. CD14+ monocytes isolated from blood of healthy donors were differentiated to immature DCs with GM-CSF and IL-4 for 5 days. Immature DCs were then stimulated with low-dose LPS, followed by microbiome-derived peptides or $OVA_{323-339}$ peptide as control. Cell supernatants were collected after 24 hours and levels of TNF-α, CXCL10, IL-6, and IFN-γ were measured by Meso Scale Discovery biomarker assays. **(B)** CXCL10 secretion levels in human DCs supernatants induced by microbiome-derived peptides (average of 2 donors). Results are shown in log2 fold-change relative to $OVA_{323-339}$ peptide control. Peptide hits were selected based on selection criteria outlined. SG-3-00802 induces CXCL10 at > 4-fold over $OVA_{323-339}$ control in 2 tested donors. **(C)** Dose response of SG-3-00802 in low-dose LPS-stimulated human monocyte-derived and mouse bone marrow-derived DCs.

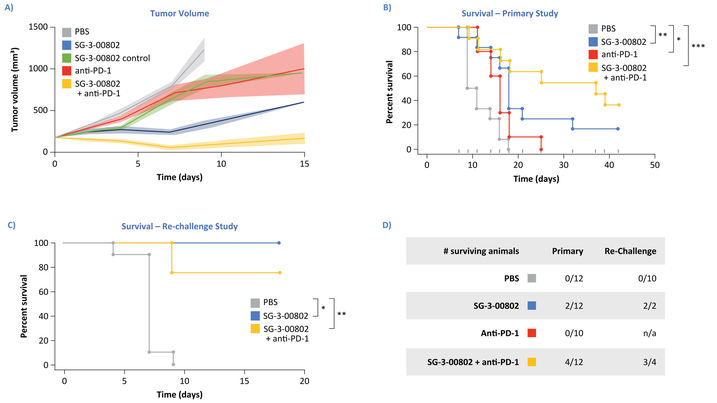
FIGURE 3:
SG-3-00802 increases CXCL10 levels and lymphocyte infiltrates in the tumor microenvironment



CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; NK, natural killer; PBS, phosphate-buffered saline.

Figure 3. Female Balb/c mice (n > 10/group, 8 weeks of age) were inoculated subcutaneously with 1×10^6 RENCA renal adenocarcinoma cells. When tumors reached a volume of > 100 mm^3 (d0), (i) mice were treated with SG-3-00802 administered peritumorally (p.t.) twice daily at 2.5mg/kg, (ii) anti-PD-1 was administered intraperitoneally (i.p.) twice weekly at 10mg/kg, (iii) or control peptide SG-3-04785 was administered p.t. twice daily at 2.5mg/kg. On day 7 after treatment, serum and tumors were collected. **(A)** Analysis of CXCL10 protein levels in serum of RENCA tumor–bearing mice 2 h after treatment with the last dose of SG-3-00802. Data show values for individual mice with the median. Statistical significance was determined by one-way ANOVA. **(B)** Analysis of CXCL10 and CD8a gene expression (relative to β-actin) in tumors of RENCA tumor–bearing mice 2 h after treatment with the last dose of SG-3-00802. Data show values for individual mice with the median. Statistical significance determined by one-way ANOVA. **(C)** Tumors were excised 2 h after treatment with the last dose of SG-3-00802 and dissociated into single-cell suspensions using the Miltenyi Tumor Dissociation Kit, and then used for flow cytometry-based analysis. CD8 T lymphocytes were gated as CD3+ CD8+, and NK cells were gated as CD3- Nkp46+. Data show values for individual mice with the median. Statistical significance was determined by one-way ANOVA.

FIGURE 4:
SG-3-00802 induces tumor regression and improves survival and inhibits tumor regrowth in an anti-PD-1-resistant model



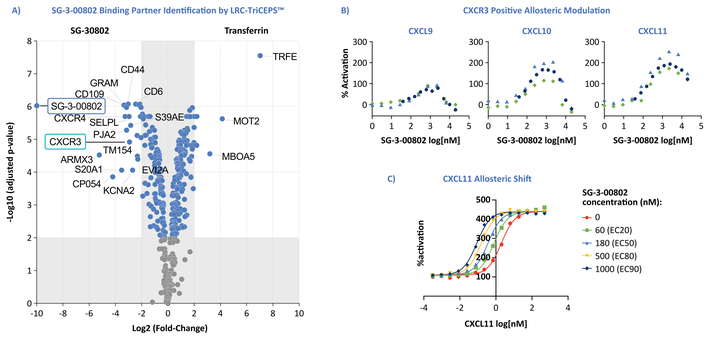
D)

# surviving animals	Primary	Re-Challenge
PBS	0/12	0/10
SG-3-00802	2/12	2/2
Anti-PD-1	0/10	n/a
SG-3-00802 + anti-PD-1	4/12	3/4

PBS, phosphate buffered saline; PD-1, programmed death-1; SEM, standard error of the mean.

Figure 4. Balb/c mice (n > 10/group, 8 weeks of age) were inoculated subcutaneously with 1×10^6 RENCA renal adenocarcinoma cells. When tumors reached a volume of ~100 mm^3 (d0), (i) mice were treated with SG-3-00802 administered peritumorally (p.t.) twice daily at 2.5mg/kg, (ii) anti-PD-1 administered intraperitoneally (i.p.) twice weekly at 10mg/kg, (iii) a combination of SG-3-00802 + anti-PD-1. Control mice were injected twice daily p.t. with corresponding volume of PBS. For re-challenge studies, animals that remained tumor free after the primary implant and treatment were newly implanted with RENCA tumors but did not receive further treatment. Tumor-naive mice were used as controls. Tumor growth and survival were monitored until the experimental endpoints. **(A)** Tumor growth of mice treated with SG-3-00802 ± anti-PD-1. Data represent mean tumor volumes ± SEM. Adjusted p-value = ****p < 0.0001; statistical significance determined by mixed-effect modeling type II ANOVA with Holm multiple comparisons test. **(B, C)** Survival was monitored until the experimental endpoints: tumor volume > 1000mm^3 ulceration. Statistical differences in survival were assessed by a Mantel-Cox log-rank test (*p < 0.05, **p < 0.01, ***p < 0.001). Data analysis of *in vivo* tumor studies was performed using the R package *TumGrowth*. **(D)** The table summarizes number of animals with complete tumor regression.

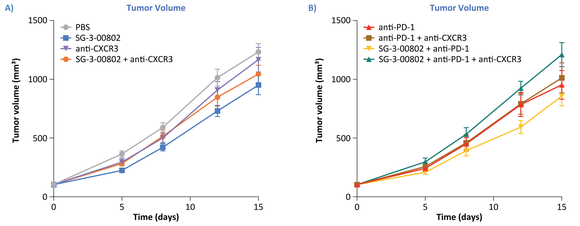
FIGURE 5:
SG-3-00802 is a positive allosteric modulator of CXCR3



CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; FRET, Förster resonant energy transfer; LRS, ligand-based receptor capture; TRFE, transferrin.

(A) SG-3-00802 binding partners were identified using the mass spectrometry-based proteomics (LC MS/MS)-based LRC-TriCEPS™ technology from DualSystems. To enrich for binding partners, the ligand is coupled with the LRC-TriCEPS™ crosslinker followed by incubation with living cells expressing target receptors. The enriched proteins are purified and processed for analysis by LC-MS/MS. A parallel experiment using transferrin as control ligand enables the relative quantification of the enriched proteins. Putative binding partners of SG-3-00802 enriched over transferrin ligand are depicted in a volcano plot. Candidates that meet the following criteria for enrichment are labelled with protein IDs: Log2 fold change >2 and Log10 (adjust p-value) >2. **(B-C)** Activation of CXCR3 signaling was tested using CXCR3-bla U2OS reporter cells. Ligand binding to CXCR3 stimulates protease-tagged arrestin and recruits it to the protease site on the C-terminus of the GPCR, which triggers release of the tethered transcription factor. The free transcription factor then enters the nucleus and stimulates beta-lactamase transcription. The beta-lactamase then cleaves the substrate to provide a quantitative FRET-based readout of CXCR3 activity.

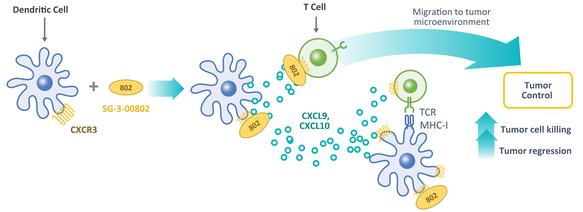
FIGURE 6:
SG-3-00802 anti-tumor activity is CXCR3-dependent



CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; PBS, phosphate-buffered saline; SEM, standard error of the mean.

Figure 6. Female Balb/c mice (n > 10/group, 8 weeks of age) were inoculated subcutaneously with 1×10^6 RENCA renal adenocarcinoma cells. When tumors reached a volume of ~100 mm^3 (d0), (i) mice were treated with SG-3-00802 administered peritumorally (p.t.) twice daily at 2.5mg/kg, (ii) anti-PD-1 administered intraperitoneally (i.p.) twice weekly at 10mg/kg, (iii) a combination of SG-3-00802 + anti-PD-1. To block CXCR3 activity, anti-CXCR3 (10mg/kg) antibody was injected twice weekly for 14 days at the same time as anti-PD-1. Control mice were injected twice daily p.t. with corresponding volume of PBS. Tumor growth of mice treated with SG-3-00802 ± anti-CXCR3 **(A)** and SG-3-00802 ± anti-PD-1 ± anti-CXCR3 **(B)**. Data represent mean tumor volumes ± SEM.

FIGURE 7:
SG-3-00802-mediated activation of CXCR3 – A promising approach to improve cancer therapy



*Proposed mechanism of action (MOA) based on target identification along with *in vitro* and *in vivo* functional assays.
CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; TCR, T cell receptor; MHC-I, major histocompatibility complex class 1.

Figure 7. Proposed mechanism of SG-3-00802-mediated activation of CXCR3. SG-3-00802 binds to CXCR3 on dendritic cells inducing endogenous CXCL10 ligand release and allosteric activation of CXCR3 to its ligands. Activation of CXCR3 leads to increased effector recruitment and contributes to T cell - dendritic cell interactions in the tumor to further enhance anti-tumor activity leading to tumor cell killing and tumor regression.

DISCUSSION

- The microbiome-derived peptide, SG-3-00802 demonstrates enhanced anti-tumor preclinical activity in combination with anti-PD-1 antibody by potentiating a robust immune response through the allosteric activation of the CXCR3 receptor.
- SG-3-00802 potentially allows for a novel mechanism to enhance the CXCL9/10/11-CXCR3 axis activation demonstrated to be critical for ICI activity in previous studies.[4]
- Based on the encouraging *in vitro* and *in vivo* data, SG-3-00802 has been selected as a lead candidate for further characterization and development.
- By enhancing antigen presentation and effector cell recruitment in lymphoid tissues and the tumor microenvironment, SG-3-00802 drives complimentary mechanisms to other immunotherapies, such as checkpoint inhibitors, cytokines, innate immune activators, and adjuvants. This offers additional potential for combination therapies not only with immunomodulators, but also with chemotherapy or radiotherapy.[5,6]

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Disclosures

Dr. Kiefel is an employee of Second Genome Inc.

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