Immune-12, a marine-derived nutraceutical, exhibits anti-glioblastoma activity in vitro and in vivo with minimal toxicity

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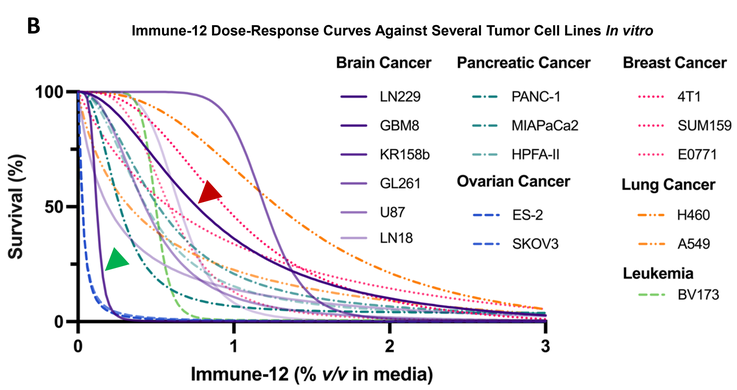
Abstract

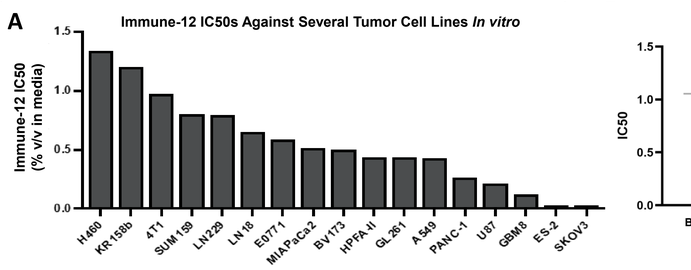
Glioblastoma (GBM) remains one of the most lethal and treatment-resistant cancers, underscoring the urgent need for novel therapeutic strategies. Marine-derived nutraceuticals offer a promising avenue due to their unique and myriad bioactive compounds. Immune-12 is an orally available marine organism-derived agent that has shown preliminary evidence of pre-clinical anticancer activity. Now, our team is conducting a comprehensive investigation of Immune-12 to characterize its molecular makeup, mechanisms of action, and *in vitro/in vivo* antitumor potencies. We have already conducted proteomic, peptidomic, and metabolomic profiling of this complex compound alongside several *in vitro* and *in vivo* efficacy and safety studies. *In vitro* cytotoxicity assays across 18 tumor cell lines, including six GBM lines, revealed potent activity, with IC_{50} values ranging from 0.1% to 1.4% Immune-12 in media (volume/volume). *In vivo* toxicity studies, in which healthy mice were given Immune-12 for 28 days via oral gavage, showed no signs of systemic toxicity or weight loss. Furthermore, in orthotopic tumor-bearing mouse models, including GBM, Immune-12 inhibited tumor growth as a monotherapy and enhanced the antitumor efficacy of standard therapeutics in combination regimens. These findings suggest that Immune-12 exerts broad-spectrum cytotoxic effects against tumor cells while maintaining a favorable safety profile *in vivo*, highlighting its potential as a marine-derived therapeutic candidate for GBM and other malignancies. Based on these promising early data, we are rapidly conducting further experiments to maximize its potential for clinical application.

Figure 1: Immune-12. (A) Immune-12 is an orally available marine-derived agent packaged in 20ml packets. **(B)** Deconvoluted mass spectra of multiple packets from multiple batches of Immune-12 show similarity in proteomic profile. **(C)** Proteins in Immune-12 identified by UniProt analysis show components from sea cucumber, sea urchin, sea squirt, and seaweed.

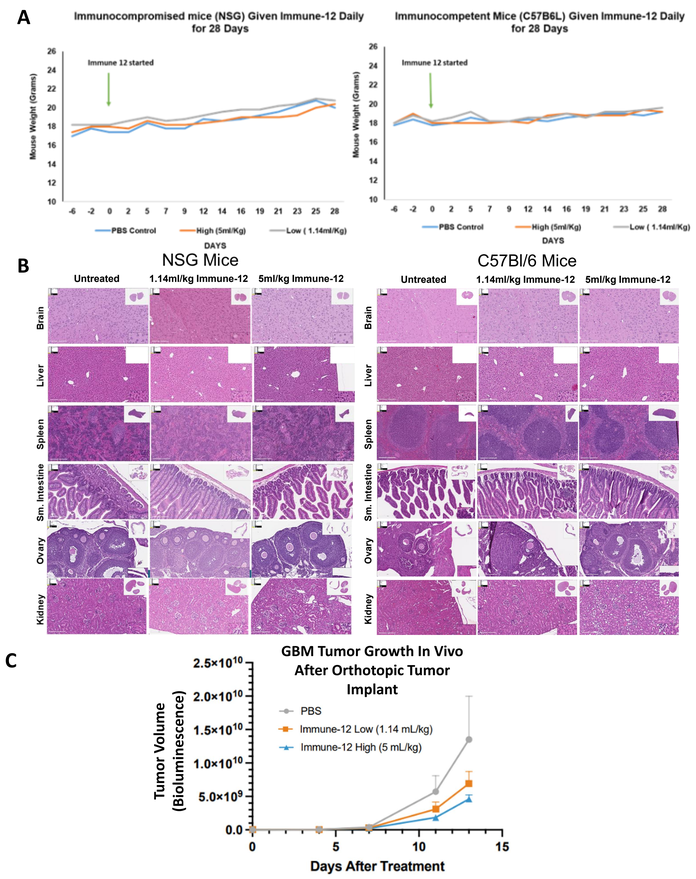
In Vitro

Figure 2: In Vitro Studies. (A-B) Immune-12 shows selective killing of a wide range of brain tumor and non-brain tumor cell types. **(C)** Immune-12 shows lower toxicity to healthy brain tissue (rat-derived organotypic brain slice cultures) than to tumor cell lines. **(D)** At 0.5% Immune-12 in media, sensitive GBM8 shows upregulations in caspase-mediated apoptosis and inductions in several apoptotic pathways, but more resistant LN229 does not. **(E-F)** Average IC50 vs LN229 across five packets from eight batches is ~0.9% v/v in media. **(G)** Immune-12 activity exists in both soluble and insoluble fractions, as quantified by gradual decreases in potency against LN229 cells.



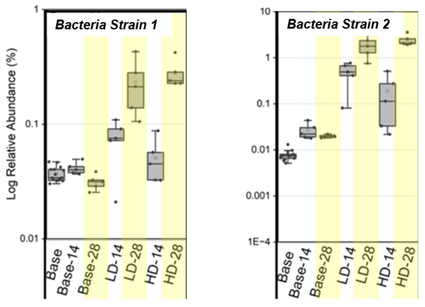


In Vivo



Examples of Gut Microbiome Changes After Daily Immune-12 Dosing in C57Bl/6 Mice

Examples of changes in bacterial abundances among all groups



Differences in alpha diversity of enzyme concentrations among all groups

Bonferroni Test

	Mean	Groups
C57B6L_Control_28	0.92805	A
C57B6L_Control_14	0.91983	A
C57B6L_LD_14	0.91898	A
C57B6L_HD_14	0.75751	A B
NSG_HD_28	0.71106	A B
C57B6L_Baseline_6	0.68834	A B
NSG_LD_28	0.67792	A B
C57B6L_LD_28	0.6598	A B
NSG_HD_14	0.64736	A B
NSG_LD_14	0.62396	A B
NSG_Control_14	0.59333	B
NSG_Control_28	0.56765	B
NSG_Baseline_6	0.56053	B
C57B6L_HD_28	0.55517	B

Means that do not share a letter are significantly different.

Fig 3: In Vivo Studies. (A-B) Immune-12 is well-tolerated in healthy mice. Mice given 1.14ml/kg/day or 5ml/kg/day of Immune-12 by oral gavage for 28 consecutive days showed no change in body weight relative to controls, nor any observable lethargy, changes in activity, seizures or abdominal pain. Histology of mouse organs after study completion revealed few changes after treatment; high-dose Immune-12 did increase white pulp in spleens. **(C)** In NSG mice with GL261 glioblastoma tumor cells implanted orthotopically in the brain parenchyma, Immune-12 induced promising trends in tumor growth inhibition. **(D)** Microbiome analysis of fecal samples of mice given 1.14ml/kg/day or 5ml/kg/day Immune-12 for 14 or 28 consecutive days also showed several significant upregulations in beneficial bacterial strains and significant changes in the alpha diversity of enzymes.

Conclusions

Our investigations around Immune-12 and its potential as an anticancer agent have generated promising early results. Immune-12 has a complex but reproducible physical makeup that potently and reproducibly kills tumor cells in vitro via caspase-mediated apoptosis. Immune-12 is also well-tolerated in mice and is showing signs of tumor growth inhibition in orthotopic models of glioblastoma. Immune-12's effects on the murine microbiome are also significant, and we are currently conducting studies to determine whether these changes can positively influence tumor kill *in vivo*. Such results would suggest a second, indirect mechanism of action of Immune-12, where the product induces changes to the body, and those changes subsequently impact tumor growth.

Support

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