The ability of HIV to establish a long-lived latent infection within resting CD4+ T cells leads to persistence and episodic resupply of the virus in patients treated with antiretroviral therapy (ART), thereby preventing eradication of the disease. Bryostatin can activate these latently infected cells, potentially leading to their elimination by virus-mediated cytopathic effects, the host's immune response and/or therapeutic strategies targeting cells actively expressing virus (see e.g., Marsden et al., "In vivo activation of latent HIV with a synthetic bryostatin analog effects both latent cell "kick" and "kill" in strategy for virus eradication", *PLOS Pathogens* 13(9): e1006575). Elimination of latently infected cells when done in conjunction with ART to eliminate the active virus, represents a strategy for treatment interruption or eradication of the disease. Elimination of latently infected cells when done in conjunction with broadly neutralizing antibodies (bNAbs) to eliminate the active virus, may also represent a strategy for treatment interruption or eradication of the disease (see e.g., Borducchi et al., "Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys," *Nature* (2008) Nov;563(7731): 360-364).

SUMMARY

This disclosure provides the use of bryostatin 1 and analogs based on the bryostatin scaffold ("bryostatin agents") as cell modulating agents. The subject bryostatin agents can be used to selectively enhance one or more of expression, translocation, cell surface presentation, and surface persistence, of an antigen in target cells of interest. Non-limiting examples of antigens in target cells of interest include, protein antigens, peptide antigens, neoantigens, and antigens derived from treatment of the target cells with mRNA. Provided herein are methods of modulating target cells in a subject. Examples of the methods include, administering an effective amount of a bryostatin agent to a subject to modulate immunogenicity of target cells. Examples of the subject methods include, contacting autologous or allogenic cells *ex vivo* with a bryostatin agent to modulate immunogenicity of the autologous or allogenic cells. The subject methods include, modulating the target cells for use in immunotherapy. The subject methods include, modulating the target cells for treatment of a disease. Non-limiting examples of diseases for treatment by the subject methods include, cancer, HIV, neurological disorders, dementia and Alzheimer's Disease. As such, the subject methods include a method of treating cancer, including administering to a subject an effective amount of a bryostatin agent to enhance cell surface antigen or neoantigen presentation on target cells of the subject, and administering to the subject a therapeutically

effective amount of a therapeutic agent that specifically binds the cell surface antigen to treat the subject for cancer. The subject methods may include selectively enhancing cell surface presentation of target antigens or neoantigens, and selectively decreasing cell surface presentation of other antigens or neoantigens. Examples of the subject methods also include use of the bryostatin agents to sensitize target cells to clearance by the subject's immune system.

In one aspect, the present disclosure relates to an *ex vivo* method of modulating target cells, the method comprising contacting target cells with an effective amount of a bryostatin agent to selectively enhance one or more of a) expression of an antigen in the target cells, b) translocation of an antigen in the target cells, c) cell surface presentation of an antigen in the target cells, and d) cell surface persistence of an antigen in the target cells, to modulate immunogenicity of the target cells.

In another aspect, the present disclosure relates to a use of a bryostatin agent and a therapeutic agent in the manufacture of a medicament for treating a subject for cancer, wherein the bryostatin agent enhances cell surface antigen or neoantigen presentation and persistence on target cells of the subject; and the therapeutic agent specifically binds the cell surface antigen to treat the subject for cancer.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1, panels A-D, illustrate the synthetic strategy for preparation of bryostatin and analogs and binding structures with Protein Kinase C (PKC). Panel A: Retrosynthetic analysis of the bryostatin scaffold with pharmacophoric elements of the C-ring subunit identified as the C1 carbonyl, C19 hemiketal, and C26 alcohol. C13 (indicted with the sphere) is highlighted as an area of interest for analog synthesis. Panel B: Rendering of the PKC-bryostatin-membrane ternary complex. Bryostatin 1 shown inside the rectangular box. Pharmacophoric elements of the C-ring subunit of the bryostatin scaffold (see FIG. 1, panel A) interact directly with PKC (dark gray) while the A and B rings are imbedded in the plasma membrane (light gray). Panel C: Representative conformers of the bryostatin scaffold bound to PKC that fit experimentally determined intramolecular distances determined by REDOR NMR. Panel D: Convergent construction of the bryostatin scaffold from acid 1 and enal 2. C13 functionality provides a versatile starting point for late-stage diversification, avoiding interference with the pharmacophoric elements of the C ring (as identified in FIG. 1, panel A).

FIG. 2A-2B illustrates the therapeutic strategy of methods of treating cancer FIG. 2A is a schematic showing the effects of cytotoxic chemotherapeutics and FIG. 2B is a schematic showing selective mAb or CAR T-cell therapeutics.

FIG. 3, panels A-D, illustrate that CD22 site density of target cells limits CD22 CAR functionality. Panel A: Histogram of CD22 expression on CRISPR/Cas9 edited CD22 negative NALM6 B-ALL lines transduced to express varying levels of CD22. NALM6 refers to the parental cell line. Interferon gamma (Panel B) and IL-2 (Panel C) production by CD22 CAR transduced T cells upon coculture with NALM6 cell lines expressing varying CD22 site densities. *=$p<0.05$, ***=$p<0.005$, ****=$p<0.001$ by one way analysis of variance (ANOVA). Data shown in panels B and C is representative of 3 independent experiments. Lines represent means +/− standard error of measurement of triplicate wells. Panel D: Xenograft model demonstrating clearance of parental

WHAT IS CLAIMED IS:

1. An *ex vivo* method of modulating target cells, the method comprising contacting target cells with an effective amount of a bryostatin agent to selectively enhance one or more of a) expression of an antigen in the target cells, b) translocation of an antigen in the target cells, c) cell surface presentation of an antigen in the target cells, and d) cell surface persistence of an antigen in the target cells, to modulate immunogenicity of the target cells.

2. The method of claim 1, wherein the antigen is selected from a protein antigen, a peptide antigen, a neoantigen, and an antigen derived from treatment of the target cells with mRNA.

3. The method of claim 1 or 2, wherein the target cells are chimeric antigen receptor (CAR)-modified T cells or chimeric antigen receptor-natural killer cells (CAR-NK cells), and the contacting target cells with the bryostatin agent enhances expression or cell surface presentation and persistence of the CAR.

4. The method of claim 3, wherein the contacting step is performed *ex vivo* and the target cells are derived from the subject (autologous cells).

5. The method claim 3, wherein the contacting step is performed *ex vivo* and the target cells are derived from a donor (allogenic cells).

6. The method of claim 1, wherein the target cells are selected from cancer cells, cancer stem cells, and cancer progenitor cells.

7. The method of claim 6, wherein the method sensitizes the target cells to clearance by the subject's immune system.

8. The method of claim 1, wherein the target cells are HIV infected cells.

9. Use of a bryostatin agent and a therapeutic agent in the manufacture of a medicament for treating a subject for cancer, wherein the bryostatin agent enhances cell surface antigen or neoantigen presentation and persistence on target cells of the subject; and the therapeutic agent specifically binds the cell surface antigen to treat the subject for cancer.

10. The use of claim 9, wherein the subject is relapsed or refractory to targeted anticancer therapy.

11. The use of claim 9, wherein the target cancer cells present cell surface antigen on the target cell surface is at a therapeutically ineffective level before the bryostatin agent is to be administered.

12. The use of claim 9, wherein the bryostatin agent to be administered enhances one or more of a) expression of cell surface antigens, b) translocation of expressed cell surface antigens to the target cell surface, and c) persistence of cell surface antigens on the target cell surface.

13. The use of any one of claims 9-12, wherein the therapeutic agent is selected from chimeric antigen receptor expressing T cells (CAR T-cells), CAR-natural killer cells (CAR-NK cells), antibody agent, antibody drug conjugate (ADC) and bispecific antibody agent.

14. The use of any one of claims 9-13, wherein the cancer is leukemia or B cell lymphoma.

15. The use of any one of claims 9-13, wherein the cancer is melanoma, prostate cancer, breast cancer, ovarian cancer, esophageal cancer, or kidney cancer.

16. The use of any one of claims 9-15, wherein the level or expression or presentation of the cell surface antigen in target cancer cells of a sample that had been obtained from the subject is to be determined.

17. The use of any one of claims 9-16, wherein at least one additional anti-cancer therapy is to be administered the patient, wherein the additional anti-cancer therapy is selected from radiation therapy, chemotherapy, immunotherapy, checkpoint inhibitors, surgery and vasculature-targeting therapy.

18. The use of any one of claims 9-17, wherein one or more biomarkers in a sample that had been obtained from the subject is to be assessed to assay the status of the cancer.