fibroblasts, endothelial and stromal cells) that regulate surrounding cells via autocrine, paracrine or endocrine mechanisms. Immunocytokines are a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic agents to treat a variety of disease including autoimmunity and cancer. This class of biologics includes FDA-approved cytokine therapies (e.g. IFN, IL-2 and Epo) as well as an increasing number of biologics designed to block cytokine activity. The latter class of biologics includes basiliximab (IL-2R), tocilizumab and sarilumabad (IL-6R), siltuximab (IL-6), ustekinumab and its biosimilars (IL-12/IL-23 p40), secukinumab (IL-17A), bevasizumab (VEGF), and denosumab (RANKL). Pharmaceutical pipelines include an increasing number of biosimilar and biobetter molecules with sustained and targeted activities with a goal to improve drug potency, patient tolerance and clinical response.

Methods Quantitative and reproducible functional bioassays are critical for the development and manufacture of biologics drugs targeting cytokine and growth factor pathways. In many cases, existing bioassays rely on the use of primary cells and measurement of complex endpoints. These assays are highly variable, difficult to implement, and often fail to yield data quality required for drug development in a quality-controlled environment. To address this problem, we have developed a suite of bioluminescent luciferase-based reporter bioassays that can be used to quantitatively measure the activity of specific cytokines and growth factors, including: IL-2, IL-6, IL-12, IL-15, IL-17, IL-23, VEGF and RANKL.

Results These mechanism of action (MOA) reflecting bioassays exhibit the required performance metrics for use in potency and stability studies. Importantly, these bioassays have been optimized in a thaw-and-use cell format, which eliminates the need for cell culture and ensures high reproducibility, convenience and transferability.

Conclusions In summary, bioluminescent reporter-based bioassays offer significant advantages over primary cell-based bioassays and are valuable tools for the development and manufacturing of novel biologics targeting cytokine and growth factor pathways.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0843

844 IMMUNOMODULATORY ACTIVITY OF EPIGENETIC DRUGS COMBINATIONS IN MESOTHELIOMA: LAYING THE GROUND FOR NEW IMMUNOTHERAPEUTIC STRATEGIES

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Background Growing evidence are demonstrating the therapeutic efficacy of immune checkpoint inhibitors (ICI) in mesothelioma; however, a limited percentage of patients benefits from this therapeutic approach. Epigenetic modifications play a relevant role in negatively regulating the cross-talk between neoplastic and immune cells, and in contributing to the highly immunosuppressive mesothelioma microenvironment. A better understanding of mesothelioma epigenetic landscape could open the path to novel and potentially more effective approaches combining ICI and epigenetic drugs. We investigated the immunomodulatory potential of epigenetic agents by comparing the activity of DNA hypomethylating agents (DHA) with histone deacetylases inhibitors (HDACi) and EZH2 inhibitors (EZH2i), alone or combined with DHA, in mesothelioma cells.

Methods Four mesothelioma cell lines were treated with the DHA guadecitabine 1μM, or with the HDACi, Valproic Acid (VPA) 1mM, or the EZH2i, EPZ-6438 1μM, alone or combined with guadecitabine. We investigated the expression of HLA class I molecules by flow-cytometry and of PD-L1, cancer testis antigens (CTA: NY-ESO, MAGE-A1), Natural Killer Group 2 member D Ligands (NKG2DLs: MIC-A, MIC-B, ULBP2) and EMT-regulating cadherins (CDH1, CDH2) by quantitative Real-Time PCR. Fold change (FC) expression for each treatment vs untreated cells was reported as mean values (FCm) among investigated cell lines. A positive modulation of the expression was considered if FCm>1.5.

Results Guadecitabine upregulated the expression of HLA class I antigens (FCm=1.75), PD-L1 (FCm=2.38), NKG2DLs (MIC-A FCm=1.96, MIC-B FCm=2.57, and ULBP2 FCm=3.56), and upregulated/induced CTA expression. Similarly, VPA upregulated HLA class I antigens (FCm=1.67), PD-L1 (FCm=3.17), NKG2DLs (MIC-A FCm=1.78, MIC-B FCm=3.04, and ULBP2 FCm=3.75) expression; however, CTA expression was modulated only in 1 mesothelioma cell line. Conversely, EPZ-6438 up-regulated only NY-ESO-1 and MIC-B expression in 1 mesothelioma cell line.

The addition of both VPA and EPZ-6483 to guadecitabine strengthened its immunomodulatory activity. Specifically, guadecitabine plus VPA or EPZ-6438 upregulated the expression of HLA class I antigens FCm=2.55 or 2.69, PD-L1 FCm=8.04 or 2.65, MIC-A FCm=3.81 or 2.26, MIC-B FCm=8.00 or 3.03, ULBP2 FCm=6.24 or 4.53, respectively. Higher levels of CTA upregulation/induction were observed with combination treatments vs guadecitabine alone.

Cadherins modulation was mesothelioma histotype-related: CDH1 expression was induced in the 2 constitutively-negative sarcomatoid mesothelioma cells by guadecitabine alone or combined with VPA or EPZ-6438; CDH2 expression was upregulated by VPA alone (FCm=1.53) or plus guadecitabine (FCm=2.54).

Conclusions Combination of DHA-based immunotherapies with other classes of epigenetic drugs could be an effective strategy to be pursued in the mesothelioma clinic.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0844

845 DEVELOPING MORE POTENT INHIBITORS OF VASOACTIVE INTESTINAL PEPTIDE SIGNALING WITH ENHANCED EFFICACY IN MOUSE MODELS OF LEUKEMIA

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Background Vasoactive intestinal peptide (VIP) is an immunosuppressive neuropeptide that significantly affect proliferation and anti-tumor properties of T cells.[1-3] VIP overexpression is a potential mechanism of immune escape in solid tumors with paracrine VIP production. Our published work shows that inhibiting VIP receptor (VIP-R) signaling via VIPhyb, an antagonistic fusion peptide between neurotensin and VIP, improves T cell dependent anti-tumor response in mouse models of acute myeloid leukemia (AML) and T lymphoblastic leukemia

(TLL).[4] In this study, we developed novel VIP-R antagonists with enhanced efficacy when compared to VIPhyb, to generate a significantly more robust anti-tumor response in mouse models of AML.

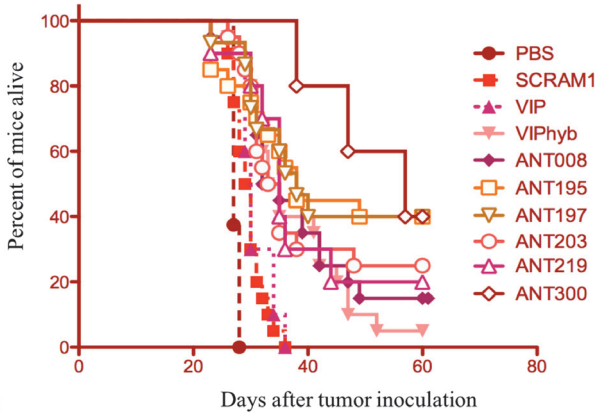
Methods We created a combinatorial library of 300 peptide sequences that contain the six charged N-terminal residues of the neurotensin present in VIPhyb (first-generation VIP antagonist) with two or more amino acid substitutions within the C-terminal amino acid sequence of VIP (table 1). We performed in-silico screening to identify 10 novel VIP-R antagonists that were predicted to have increased binding affinity to VIP receptors VPAC1 and VPAC2 when compared to VIP or VIPhyb. The efficacy of these peptides where tested in-vitro using T cells from luciferase transgenic mice seeded and expanded on anti-CD3 monoclonal antibody coated plates for three days. Enhanced potency of the novel antagonists in vivo, was tested in a mouse AML model, by treating C1498-bearing mice with subcutaneous administration of VIP, VIPhyb, scrambled peptide or the second-generation VIP-R antagonists (labeled as 'ANT') from day 6-12 after tumor implantation.

Results T cell proliferation using 0.3 µM of a novel VIP-R antagonist was increased up to 216% + 20% of control cultures without added peptides versus 197% + 38% in cultures with VIPhyb at 1 uM (table 1). Furthermore, the novel VIP-R antagonists increased median survival times (MST) by up to 57 days and rendered 40% of mice leukemia-free at 60 days compared to MST of 34 days and 5% long-term survival with VIPhyb (figure 1).

Conclusions In this study, for the first time, we have identified novel and more potent VIP-R antagonists when compared to VIPhyb, with enhanced potency to activate and proliferate T cells and generate an effective anti-tumor response in mouse models of leukemia. These novel antagonists can lead to peptide-based immunotherapy for the treatment of various solid and liquid cancers, such as the cancer of the colon and pancreas, that overexpress VIP intratumorally.

Abstract 845 Table 1 Novel second generation VIP-R antagonists
Predicted binding affinity of VIP, VIPhyb and VIP antagonists to VPAC1 and VPAC2 based upon in silico screening is shown along with the proliferation of luciferase+ mouse T cells and their anti-leukemia activity in mice. The level of T cell bioluminescence vs. control T cells stimulated with anti-CD3 alone is shown along with the lowest peptide concentration that achieved the maximal effect on T cell proliferation. Median survival times and the fraction of mice alive at day 60 along with the numbers of animals tested with each VIP antagonist are shown.

Peptide name	Docking score VPAC1-R (Kcal/mol)	Docking score VPAC2-R (Kcal/mol)	Relative T cell proliferation vs no peptide control and most effective concentration	Percentage of mice alive at day 60 and median survival times (MST)
VIP	-65.8	-52.6	77% ± 13% (0.1µM)	0%, MST 30 days, p=0.328, n=10
VIPhyb	-60.2	-51.01	197% ± 38% (1µM)	5%, MST 34 days, p=0.0001, n=20
SCRAM1	-42.84	-37.10	109% ± 8% (3µM)	0%, MST 29.5 days, n=20
ANT005	-64.27	-64.45	187% ± 1% (3µM)	Pending
ANT008	-60.17	-53.98	185% ± 24% (1µM)	15% MST 33.5 days, p=0.0002, n=20
ANT058	-76.3	-60.60	pending	Pending
ANT105	-68.19	-55.35	121% ± 6% (3µM)	Pending
ANT195	-70.44	-71.44	161% ± 23% (3µM)	40%, MST 38 days, p<0.0001, n=20
ANT197	-63.6	-69.07	161% ± 13% (3µM)	40%, MST 38 days, p<0.0001, n=15
ANT202	-56.35	-67.02	171% ± 8% (3µM)	Pending
ANT203	-75.34	-50.94	185% ± 8% (3µM)	25%, MST 34 days, p=0.0002, n=20
ANT219	-69.37	-69.12	189% ± 8% (3µM)	20%, MST 35 days, p=0.0009, n=10
ANT300	-72.6	-61.6	216% ± 20% (0.3µM)	40%, MST 57 days, p=0.0001, n=5



Abstract 845 Figure 1 Prolonged survival with second generation peptides
C57BL/6 mice were injected intravenously with 1 x 106 C1498 myeloid leukemia cells on day 0. The presence of engrafted was confirmed on day 6 by flow cytometry and leukemia-bearing mice were treated with daily subcutaneous injections of PBS, 10 µg VIP scrambled peptide (SCRAM), VIP, VIPhyb, or a second-generation VIP antagonist (ANT008, etc…) for 10 days. Survival of all groups treated with a VIP antagonist was significantly better than groups treated with PBS, VIP, or SCRAM at $p < 0.001$. Survival between mice treated with ANT300 was significantly better than those treated with VIPhyb ($p<0.02$)

REFERENCES

1. Gonzalez-Rey E, Anderson P, Delgado M, Emerging roles of vasoactive intestinal peptide: a new approach for autoimmune therapy. *Ann Rheum Dis* 2007;**66**(Suppl 3):iii70-6.
2. Anderson P, Gonzalez-Rey E. Vasoactive intestinal peptide induces cell cycle arrest and regulatory functions in human T cells at multiple levels. *Mol Cell Biol* 2010;**30**(10):2537-51.
3. Reubi JC, *et al*. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor subtypes in human tumors and their tissues of origin. *Cancer Res* 2000;**60**(11):3105-12.
4. Petersen CT, Li JM, Waller EK. Administration of a vasoactive intestinal peptide antagonist enhances the autologous anti-leukemia T cell response in murine models of acute leukemia. *Oncoimmunology* 2017;**6**(5):e1304336.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0845

846 NEOSPORA CANINUM – AN IMMUNOTHERAPEUTIC PROTOZOAN AGAINST SOLID CANCERS

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Background Immunotherapy induces, provides, and/or reactivates anti-tumor immune responses. Some microorganisms also can initiate response that lyzes infected tumor and/or stimulates systemic immunity. Attenuated viruses or bacteria are well studied as oncotherapeutics, but not protozoa except Toxoplasma gondii.[1] We assessed the effect on tumors of other protozoa that were naturally non-pathogenic to humans. Thus, we discovered the ability to use Neospora caninum (Nc) in a manner and form that demonstrated a synergistic array of pertinent immunotherapeutic characteristics against solid cancers. Our first Article on Neospora as Onco-immunotherapeutic is currently under revision after review by the JITC. We report on the most recent data notably from Nc engineered to secrete human IL-15 within the tumor.

Methods In vitro, the immunostimulatory properties of Nc strains wildtype and engineered to secrete human IL-15 were