fibroblasts, endothelial and stromal cells) that regulate surrounding cells via autocrine, paracrine or endocrine mechanisms. Immune cytokines 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 increasing number of biologics designed to block cytokine activity. The latter class of biologics includes basiliximab (IL-2R), tocilizumab and sarilumab (IL-6R), silixumab (IL-6), ustekinumab and its biosimilars (IL-12/IL-23 p40), secukinumab (IL-17A), bevacizumab (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.

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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 neo- plastic 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 testsis 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-6438 to guadecitabine strengthened its immunomodulatory activity. Specifically, guadecitabine plus VPA or EPZ-6438 upregulated the expression of HLA class I antigens FCm=2.53 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.

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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 VIPthb, 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.
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 μM (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.

**REFERENCES**


**Abstracts**

**Table 1** Novel second generation VIP-R antagonists

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Dangling score (VPAC1 Vs VPAC2)</th>
<th>Dangling score (VPAC1 Vs VPAC2)</th>
<th>Relative T cell proliferation vs no peptide control and most effective concentrations</th>
<th>Percentage of mice alive at day 60 and median survival times (MST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>60.0</td>
<td>60.0</td>
<td>77% vs 19% (4x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
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<tr>
<td>VIPhyb</td>
<td>60.0</td>
<td>60.0</td>
<td>197% vs 19% (4x)</td>
<td>1% MST 30 days, p=0.001, n=20</td>
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<tr>
<td>SCRAM1</td>
<td>60.0</td>
<td>50.0</td>
<td>100% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
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<tr>
<td>ANT008</td>
<td>60.0</td>
<td>60.0</td>
<td>143% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
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<tr>
<td>ANT195</td>
<td>60.0</td>
<td>60.0</td>
<td>166% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
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<tr>
<td>ANT203</td>
<td>60.0</td>
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<td>225% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
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<tr>
<td>ANT219</td>
<td>60.0</td>
<td>60.0</td>
<td>280% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
</tr>
<tr>
<td>ANT300</td>
<td>60.0</td>
<td>60.0</td>
<td>340% vs 19% (5x)</td>
<td>1% MST 30 days, p=0.001, n=10</td>
</tr>
</tbody>
</table>

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.