Abstracts

(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 were 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 μ M 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 μ M (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% $\pm$ 13% (0.1 μ M)	0%, MST 30 days, p=0.328, n=10
VIPhyb	-60.2	-51.01	197% $\pm$ 38% (1 μ M)	5%, MST 34 days, p=0.0001, n=20
SCRAM1	-42.84	-37.10	109% $\pm$ 8% (3 μ M)	0%, MST 29.5 days, n=20
ANT005	-64.27	-64.45	187% $\pm$ 1% (3 μ M)	Pending
ANT008	-60.17	-53.98	185% $\pm$ 24% (1 μ M)	15% MST 33.5 days, p=0.0002, n=20
ANT058	-76.3	-60.60	pending	Pending
ANT105	-68.19	-55.35	121% $\pm$ 6% (3 μ M)	Pending
ANT195	-70.44	-71.44	161% $\pm$ 23% (3 μ M)	40%, MST 38 days, p<0.0001, n=20
ANT197	-63.6	-69.07	161% $\pm$ 13% (3 μ M)	40%, MST 38 days, p<0.0001, n=15
ANT202	-56.35	-67.02	171% $\pm$ 8% (3 μ M)	Pending
ANT203	-75.34	-50.94	185% $\pm$ 8% (3 μ M)	25%, MST 34 days, p=0.0002, n=20
ANT219	-69.37	-69.12	189% $\pm$ 8% (3 μ M)	20%, MST 35 days, p=0.0009, n=10
ANT300	-72.6	-61.6	216% $\pm$ 20% (0.3 μ M)	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 μ g 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)

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NEOSPOA CANINUM – AN IMMUNOTHERAPEUTIC PROTOZOAN AGAINST SOLID CANCERS

¹Louis Lantier, ¹Agathe Poupee-Beauge, ¹Louis Lantier*, ¹Céline Ducournau, ¹Anne Di Tommaso, ¹Stéphanie Germon, ¹Nathalie Moiré, ²Gordon Lee, ¹Antoine Touze, ¹Isabelle Dimier-Poisson. ¹Université de Tours, Tours, France; ²Kymeris Therapeutics, Toronto, Canada

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

studied. In vivo experiments of treatment with of *Nc* tachyzoites² administered locally (intra and peri tumoral) or remotely (subcutaneous) in a murine thymoma EG7 tumor and in human Merkel cell carcinoma (MCC).

Results We demonstrated that the treatment of thymoma EG7 by *Nc* strongly inhibited tumor development. Analysis of immune responses and interactions between *Nc* and tumor cells showed that *Nc* had the ability to lyse infected cancer cells, reactivated immune competence within the Tumor Microenvironment (TME), and activated the systemic immune system by promoting the recruitment of immune cells to the site of tumor. We also established in a NOD/SCID mouse model that *Nc* was able to induce a strong regression of human MCC. Recently, to further enhance oncotherapeutic effect, we engineered an *Nc* strain to secrete human IL-15 (cross reactive with mouse cells), associated with alpha subunit of IL-15 receptor, increasing its stability.³ This strain induced proliferation of human PBMCs and their secretion of IFN- γ . In the EG7 model, human IL-15 secreting *Nc* showed greater protection against tumor development, confirming enhancement of immunotherapy by engineering *Nc* to deliver/secrete IL-15.

Conclusions These results highlight *Neospora caninum* as a potentially extremely efficient, and non-toxic anti-cancer agent, capable of being engineered to express at its surface or to secrete bio-drugs, like human IL-15 cytokine. Our work has identified the broad clinical possibilities of using *N. caninum* as an oncolytic protozoan in human medicine capable of vectoring molecular therapy, overcoming TME defenses.

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847 INFLAMMASOME ACTIVATION IN M2 MACROPHAGE RESTRAIN THE IMMUNE SUPPRESSIVE FUNCTION

Ronghua Zhang, Tienan Wang*, Qing Lin. *WXi ApTec, Shanghai, China*

Background Macrophage is an important component in tumor microenvironment (TME) and plays multiple roles in tumor initiation, progression and metastases. In response to various stimuli within TME, macrophage exhibits high level of functional heterogeneity. There are two distinct groups of macrophages: M1 macrophage exhibits pro-inflammatory phenotype with high levels of TNF- α , IL-6, and IL-1 β , while M2 macrophage displays immune suppressive phenotype with high levels of anti-inflammatory cytokines such as IL-10 and TGF- β . In response to the M2 cytokines, myeloid cells within the TME further acquire higher expression of PD-L1 and thus inactivate T cells. M2 cytokines can also directly inhibit T cell activation. As a result, re-polarizing M2 macrophages becomes a key concept for cancer immunotherapy. The NLRP3 inflammasome is acquired by macrophages to fight against endogenous danger signals. Macrophage NLRP3 activation has been observed in

several tumor models, but the function of NLRP3 on macrophage polarity remains controversial. Inflammasome activation with IL-1 β /IL-18 secretion was reported to promote M1 polarization. However, NLRP3 activation was also reported to promote M2 polarity through up-regulation of IL4 in asthma model

Methods Here, we have established an in vitro human macrophage NLRP3 activation system (figure 1), coupled with M2 macrophage polarization assay, to dissect the role of NLRP3 in macrophage phenotype.

Results Our results indicate that NLRP3 activation restrained M2 phenotype and further enhanced T cell activation in an M2/T cell co-culture system (figure 2).

Abstract 847 Figure 1 Inflammasome activation polarize M2 macrophage int

Use LPS/ATP to stimulate NLRP3 in M2 macrophage and demonstrate NLRP3 activation could reduce CD163 and increase CD86

Abstract 847 Figure 2 Inflammasome in M2 rescue T cell activation establish M2/T co-culture system in vitro to demonstrate M2 could suppress T activation while Inflammatory M2 could partial rescue the suppressive phenotype

Conclusions Inflammasome could be the potential target for cancer by modulating T cell activation through macrophage polarization regulation

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