491

NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy

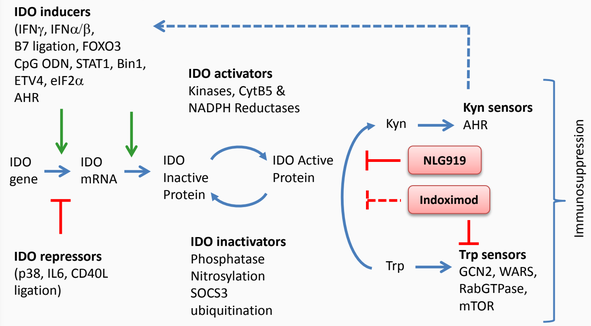
M. Mautino[1], F. Jaipuri[1], J. Waldo[1], S. Kumar[1], J. Adams[1], C. Van Allen[1], A. Marcinowicz-Flick[1], D. Munn[2], N. Vahanian[1] and C. J. Link[1]

[1] NewLink Genetics Corporation, Ames, IA ; [2] Georgia Regents University, GA

Introduction

- The IDO pathway mediates immunosuppressive effects through the metabolization of tryptophan (Trp) to kynurenine (Kyn), triggering downstream signaling through GCN2, mTOR and AHR that can affect differentiation and proliferation of T cells.
- Expression of the IDO1 gene by tumor cells or host APCs can inhibit tumor-specific effector CD8$^+$ T cells and enhance the suppressor activity of Tregs
- High expression of IDO correlates with worse clinical prognosis in patients with a variety of malignancies.
- Therefore, targeting the IDO pathway via inhibition of the IDO enzyme or blocking its downstream signaling effects is a prime target for small-molecule immunomodulatory drugs in cancer.

IDO Pathway



Lead Development Candidate: NLG919

Drug-Like Property		
MW		<500
LogP		1. 5
PSA		56
HBD		2
HBA		5
Rotatable bonds		3
pKa		2.6, 6.1

Property		
Human IDO IC50	nM	28
Human IDO EC50	nM	75
Mouse IDO EC50	nM	19
Cell Cytoxicity LD50	µM	>100
Ki	nM	7.2
CYP 3A4	µM	2.8
CYP 2D6	µM	69
CYP 1A2	µM	238
CYP 2B6	µM	146
hERG	µM	100
Permeability PAMPA	cm/s	-5.95
Permeability Caco-2	cm/s	22.7
PgP substrate	Efflux ratio	0.9
PPB	%Fu	52
Sol pH 1.0	mg/mL	>270
Sol pH 6.5	mg/mL	215
Sol pH 7.4	mg/mL	213

DMPK-Tox

Pharmacokinetics

		Mouse	Rat			Dog			
Dose	mg/kg	10	10	25	50	5	15	50	150
C_{max}	ng/mL	2300	1560	6420	10050	127	1395	9485	40175
$AUC_{(0-\infty)}$	ng*h/mL	3792	1630	7975	15400	212	1073	9380	54525
$T_{1/2}$	h	1	1.1	1.1	1.1	5.5	3	3.9	4.7
MRT	h	1.9	1.1	1.4	1.5	6.6	2.8	2.2	2.1
% F	%	71	41	64	45	13	21	53	90
Cl	mL/kg/min	31	45	32	23	53	41		
Vd_{ss}	L/kg	2.3	2.3	2.0	1.5	2.8	2.8		

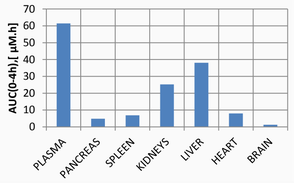
Toxicology

Genetic Toxicology	Species	Max Dose	Result	
Ames Test	Bacteria	5 mg/plate	Negative	
Chromosomal Aberrations	Hamster (CHO)	317 µg/mL	Negative	
Bone Marrow Micronucleus	Rat	2000 mg/kg	Negative	
Dose Repeat Toxicology		**Doses (mg/kg)**	**NOAEL (mg/kg)**	**MTD (mg/kg)**
Single Dose Range finding	Rat	50, 100, 300, 600, 900	300	900
	Dog	30, 100, 200, 400, 600	200	400
7-day, bid	Rat	100, 300, 600, 1000	<300	1000
	Dog	5, 15, 50, 150	150	>150
28-day, bid	Rat	40, 80, 250	80	>250
	Dog	20, 80, 200	80	>200

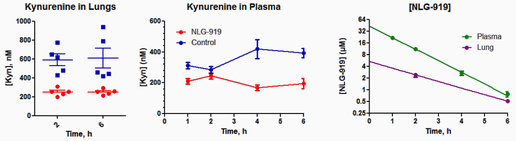
Metabolization

Metabolic Pathway	Mouse	Rat	Dog	Monkey	Human
Parent	76	50	47	84	86
Isomerization	8.4	16.9	2.3	7.1	4.8
Oxygenation	0	2.5	8.6	0.5	0
Glucuronidation	7.5	1.9	33.2	5.7	3.5
Dehydrog.	7.2	28.4	9.0	2.9	5.9
Combination	0.6	0.6	0.2	0	0

Biodistribution



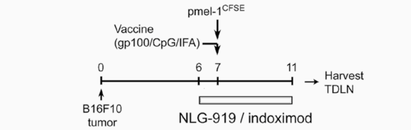
Pharmacodynamics

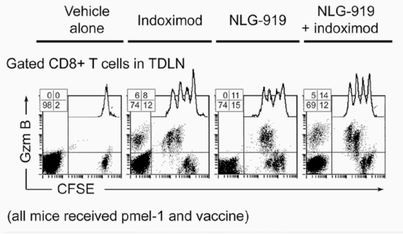


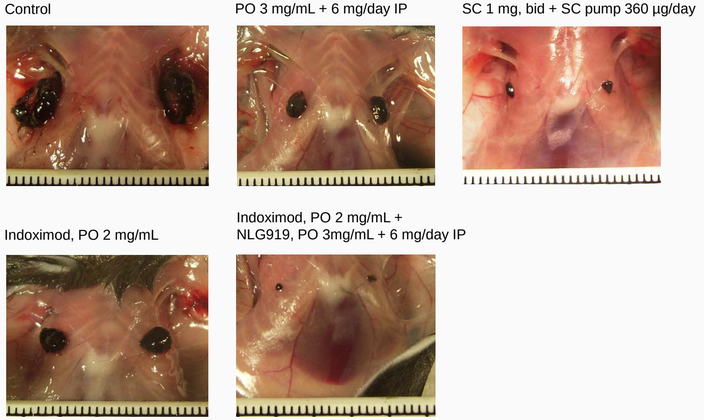
NLG919 causes reduction in kynurenine concentration.

IDO was activated by dosing mice with 25 µg of LPS intranasally 24h before plasma or tissue collection. Mice were dosed with 50 mg/kg NLG919, PO at 0h, 12h and 24h post LPS. Plasma and lung tissues were collected at different time points after the last dosing, and the concentration of Kyn and NLG919 was measured by LC/MSMS.

Antitumor Activity



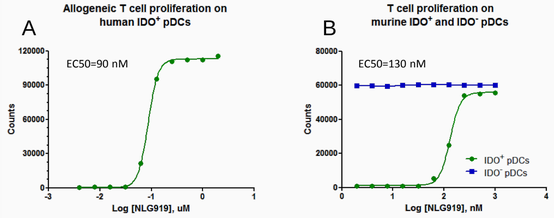




NLG919 and indoximod have synergistic antitumor activity

Mice were injected with 1x10^6 B16F10 cells on day 1. The drug administration started on day 6 for all mice except the control group. On day 7, all mice (including the control group) were injected i.v. with 2x10^6 CFSE-labeled pmel-1 CD8$^+$ cells (isolated by magnetic beads from spleens of pmel-1 mice) and vaccinated in the footpad with an emulsion of gp100 peptide (KVPRNQDWL, 25 µg) and CpG1826 ODN in IFA. Tumors were evaluated at day 11 after 4 days of drug treatment. Indoximod was dosed dissolved in the drinking water at 2 mg/mL. NLG919 was dosed either dissolved in the water at 3 mg/mL, plus a daily dose of 6 mg injected via IP, or administered subcutaneously at 1 mg/dose twice a day via injection plus 360 µg/day via an SC osmotic pump.

Immunoassays



NLG919 stimulates T cell proliferation in the context of allogeneic IDO$^+$ pDCs.

A) Human IDO$^+$ pDCs were generated from monocytes cultured 7d with GM-CSF, IL-4 and matured with TNFα, IL-1ß, IL-6 and PGE2. Non-adherent fraction is IDO$^+$. The graph shows [^{3}H]-Thy incorporation in a proliferation assay of allogeneic lymphocytes at a 10:1 ratio, in the presence of different concentrations of NLG919. (n=3 donors). **B)** Murine IDO$^+$ pDCs were obtained from TDLN of B16 bearing mice by sorting the CD11c$^+$/B220$^+$ cell fraction. CD8 were obtained from OT1 mice and T cell proliferation was measured by [^{3}H]-Thy incorporation in a proliferation assay, in the presence of OVA peptide (SIINFEKL). Control IDO$^-$ pDCs were used as control for cytotoxicity and proliferation.

Conclusions

- NLG919 potently inhibits the IDO pathway in vitro and in cell based assays (Ki=7 nM; EC50 =75 nM).
- NLG919 is orally bioavailable and has a favorable pharmacokinetic and toxicity profile.
- Oral administration of NLG919 reduces the [Kyn] in plasma and tissue by ~ 50%.
- Using human IDO$^+$ pDCs in allogeneic MLR reactions, NLG919 potently blocked IDO-induced T cell suppression and restored robust T cell responses with an EC50=90 nM.
- NLG919 abrogated IDO-induced suppression of antigen-specific T cells (OT-I or pmel-1) in vitro, (ED50=130 nM) using mouse IDO$^+$ pDCs from tumor-draining lymph nodes.
- In mice bearing large established B16F10 tumors, administration of NLG919 markedly enhanced the antitumor responses of naïve, resting pmel-1 cells to vaccination with cognate hgp100 peptide plus CpG-1826 in IFA. In this stringent established-tumor model, NLG919 plus pmel-1/vaccine produced a dramatic collapse of tumor size within 4 days of vaccination (~95% reduction in tumor volume compared to control animals receiving pmel-1/vaccine alone without NLG919).
- NLG919 and indoximod show synergistic T cell activation and antitumor activity.
- In conclusion, NLG919 is a potent IDO pathway inhibitor with desirable pharmacological properties, suitable for the treatment of immunosuppression associated with cancer.