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Synergistic antitumor effects of combinatorial immune checkpoint inhibition with anti-PD-1/PD-L antibodies and the IDO pathway inhibitors NLG919 and indoximod in the context of active immunotherapy

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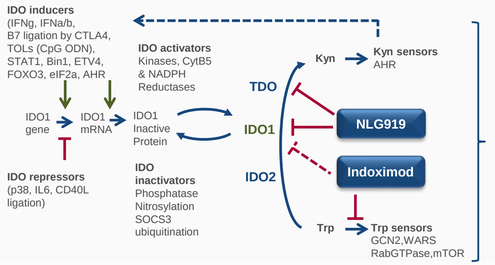
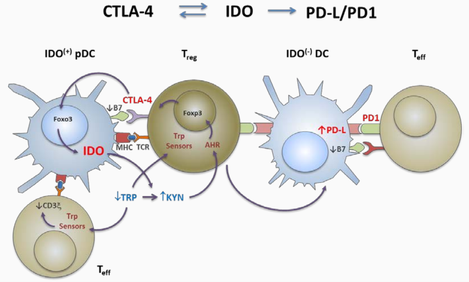
Introduction

- The IDO pathway mediates immunosuppressive effects through the metabolization of tryptophan (Trp) to kynurenine (Kyn)[1], triggering downstream signaling through Trp sensors GCN2[2] and mTOR[3] and Kyn sensor AHR[4]. This signals affect differentiation of DCs and Tregs and activation and proliferation of Treg and Teff cells.[5,6]
- An active IDO pathway in tumor cells or host APCs can inhibit tumor-specific effector CD8$^+$ T cells, and enhance the suppressor activity of Tregs and DCs.
- High expression of IDO in tumor cells or APCs correlates with worse clinical prognosis in patients with a variety of malignancies.[5,7,8,9]
- NLG919 and indoximod are orally bioavailable inhibitors of the IDO pathway
- The IDO pathway is interrelated with CTLA-4 and PD-1/PD-L1 pathways
- Therefore, targeting the IDO pathway via inhibition of the IDO enzyme or blocking its downstream signaling effects in combination with other key immune checkpoint inhibitors is a prime target for small-molecule immunomodulatory drugs in cancer.

IDO Pathway

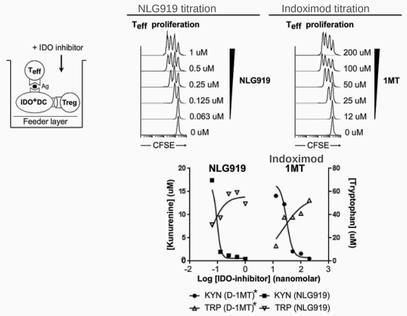


Key immune checkpoints IDO, CTLA-4 and PD-1 are interrelated



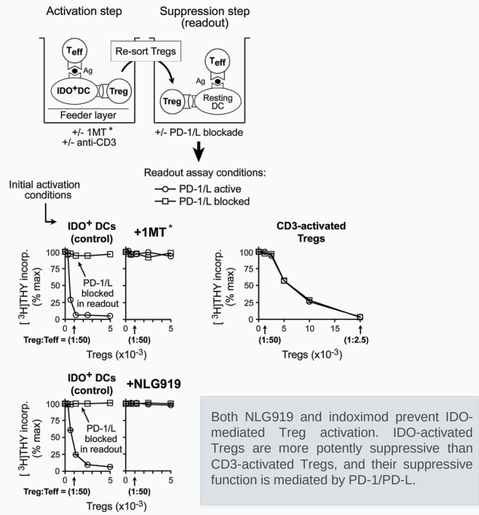
References: ***1)*** *McGaha T- Imm.Reviews 2012(249)135;* ***2)*** *Munn DH – Immunity 2005(22)633;* ***3)*** *Metz RA – Oncoimm. 2012(1)1460;* ***4)*** *Opitz CA - Nature 2011(478/7368)197;* ***5)*** *Munn DH – J. Clin. Investig. 2004(114)280;* ***6)*** *Munn DH – J. Clin. Investig. 2007(117)2570;* ***7)*** *Ferdinande L - Br J Cancer 2012(106)141;* ***8)*** *Inaba T – Gynecol Oncol 2010(117)423;* ***9)*** *Okamoto – Clin Cancer Res. 2005(11)6030.*

Stimulation of T_{eff} Cell Proliferation by IDO inhibitors



NLG919 and indoximod block IDO-mediated Trp degradation and stimulate Teff proliferation in the presence of Tregs

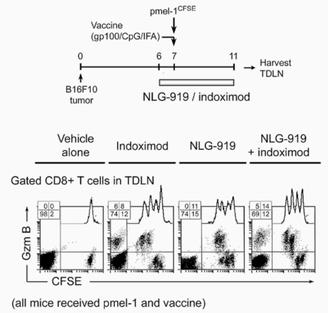
IDO-activated T_{regs} mediate suppression via PD-1/PD-L



Both NLG919 and indoximod prevent IDO-mediated Treg activation. IDO-activated Tregs are more potently suppressive than CD3-activated Tregs, and their suppressive function is mediated by PD-1/PD-L.

1MT aka indoximod

Effect of NLG919 and indoximod on T_{eff}, T_{regs} and DCs in TDLN

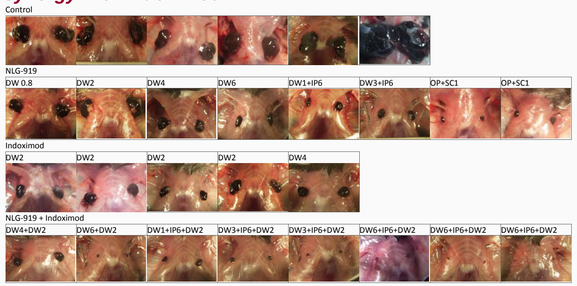


In tumor-bearing mice that receive vaccination with a tumor-associated antigen, and adoptive Teff cell transfer, NLG919 and indoximod stimulate in vivo Teff cell activation and proliferation, and mediate the reprogramming of Tregs to a T helper-like phenotype in the TDLN. At the same time they mediate the immunophenotypic conversion of CD11c$^+$ DCs from an immuno-suppressive phenotype (PD-L$^+$/B7$^-$) to an immunostimulatory phenotype (PD-L$^-$/B7$^+$).

(all mice received pmel-1 and vaccine)

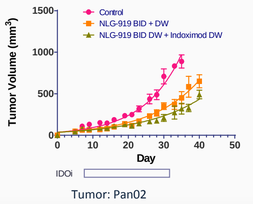
Gate	CD4$^+$ CD25$^+$ FoxP3$^+$	CD11c$^+$			
Measured parameter	Treg reprogramming	DC reprogramming			
% Phenotype (average)	CD40L (%)	PD-L1 (%)	PD-L2 (%)	CD80 (%)	CD86 (%)
Control	0	82	88	7	0
NLG919	32	19	21	55	58
Indoximod	24	11	9	83	81

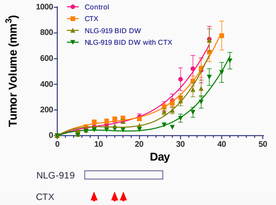
Dose dependent antitumor effect of NLG919 and synergy with indoximod



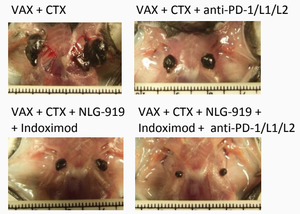
In a B16F10 tumor model, NLG919 and indoximod enhanced the antitumor responses of naïve, resting adoptively transferred pmel-1 cells to vaccination with cognate hgp100 peptide. The effect was dose-dependent for NLG919 and synergistic with co-administration of indoximod.

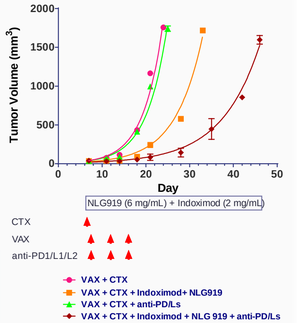
Synergistic antitumor activity of NLG919 + indoximod or chemotherapy





Synergy of IDO and PD-1/PD-L pathways inhibition





B16F10 bearing mice received CTX on d6, gp100 and a cocktail of anti-PD-1/PD-L1/PD-L2 mAb on d7, 10 and 13, and dosed with NLG919 and indoximod in drinking water from d6-d16. No adoptive pmel-1 T cell transfer.

Conclusions

- NLG919 potently blocked IDO-mediated conversion of Trp into Kyn and promoted activation and proliferation of Teff cells even in the presence of Tregs (EC50 = 125 nM). Indoximod mediated similar effects albeit at higher concentrations (EC50=33 µM).
- Activation of Tregs by IDO$^+$ DCs results in potently suppressive Tregs, which mediate their immunosuppressive effect in an IDO-independent way via PD-1/PD-L1 pathway. NLG-919 and indoximod abrogated IDO-induced activation of Tregs.
- NLG919 and indoximod are able to stimulate adoptively transferred Teff cell proliferation in TDLN, while promoting reprogramming of Tregs and DCs to an immunostimulatory phenotype, which results in a marked antitumor effect.
- In a stringent B16F10 melanoma model, a combination of immune checkpoint inhibition involving NLG919, indoximod and anti-PD-1/PD-L1/PD-L2 antibodies was synergistic compared to single checkpoint inhibition therapy.
- The current preclinical studies suggest a mechanistic rationale for a combining IDO pathway inhibitors with agents targeting the PD-1/PD-L1/PD-L2 pathway.

AACR April, 2014