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A Novel Indoximod Prodrug (NLG802) With Enhanced Pharmacokinetic Properties

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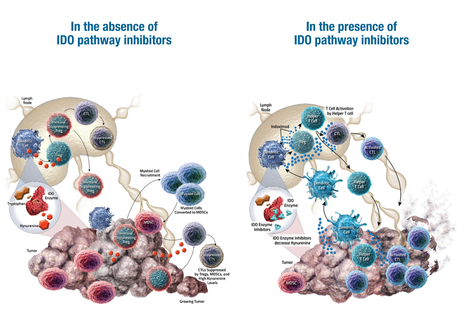
INTRODUCTION

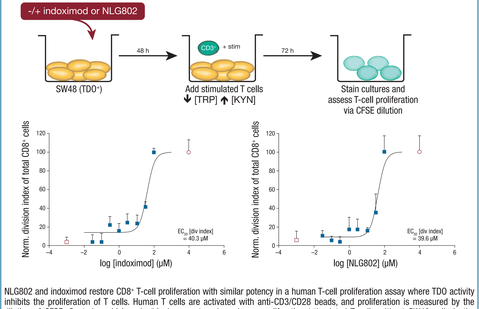
- The IDO pathway mediates immunosuppressive effects through the metabolization of tryptophan (Trp) to kynurenine (Kyn),[1] triggering downstream signaling through Trp sensors GCN2[2] and mTOR[3] and Kyn sensor AHR.[4] These signals affect the differentiation of DCs toward inhibition, activate Tregs, and modify proliferation of effector T cells[5,6] (**Figure 1**)
- TDO has the same biochemical function of IDO, and its expression in the tumor microenvironment could mimic IDO pathway effects
- An active IDO/TDO pathway in tumor cells or host APCs can inhibit tumor-specific effector CD8+ T cells and enhance the suppressor activity of Tregs and some inhibitory DC subsets. High expression of IDO in tumor cells or APCs correlates with worse clinical prognosis in patients with a variety of malignancies[5,7-9]
- Targeting the IDO/TDO pathway via inhibition of the IDO enzyme or blocking its downstream signaling effects is a prime target for small-molecule immunomodulatory drugs in cancer
- Indoximod has been demonstrated to relieve IDO-mediated immunosuppression in vitro and in vivo by the creation of an artificial Trp-sufficiency signal that bypasses activation of GCN2 and inhibition of mTOR in conditions of Trp deprivation.[3] Inhibition of the IDO pathway by indoximod in combination with immune-stimulatory treatments leads to increased T-cell proliferation, Treg reprogramming, and antitumor effect
- Indoximod has demonstrated an excellent safety profile in human clinical trials and is being dosed orally at 1200 mg bid. Increases in doses above this level do not generally result in increased plasma concentration or drug exposure due to limiting dose-dependent oral bioavailability. Animal models suggest that higher levels of exposure might increase therapeutic benefit
- NLG802 is a prodrug of indoximod that increases bioavailability and exposure of indoximod



Indoximod inhibits the effects of the IDO pathway, preventing Treg activation and MDSC recruitment, and promoting effector T-cell activation and proliferation,[10] reprogramming of Treg to TH17-helper–like T cells,[11] and dendritic cell activation.[11] Indoximod appears to activate mTOR under conditions of Trp deficiency, thus bypassing the suppressive effects of Trp starvation.[3]

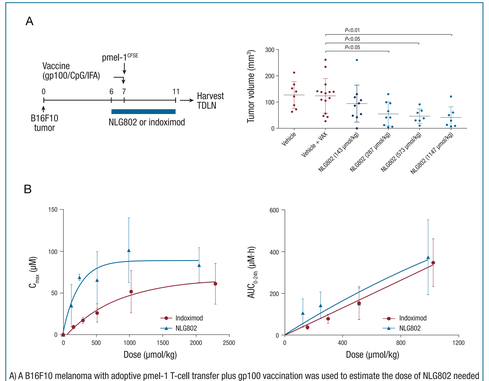
Figure 1. IDO pathway.

RESULTS



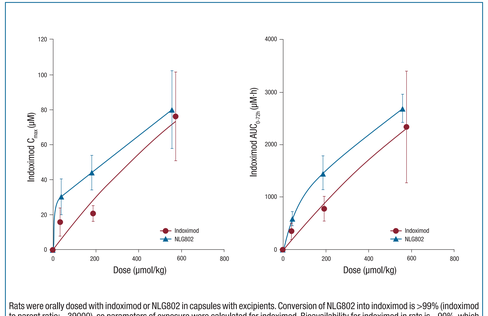
NLG802 and indoximod restore CD8+ T-cell proliferation with similar potency in a human T-cell proliferation assay where TDO activity inhibits the proliferation of T cells. Human T cells are activated with anti-CD3/CD28 beads, and proliferation is measured by the dilution of CFSE. Controls: vehicle only (blank square) and maximum proliferation (stimulated T cells without SW48 cells in the co-culture, blank circle).

Figure 2. Stimulation of CD8+ T-cell proliferation by NLG802.



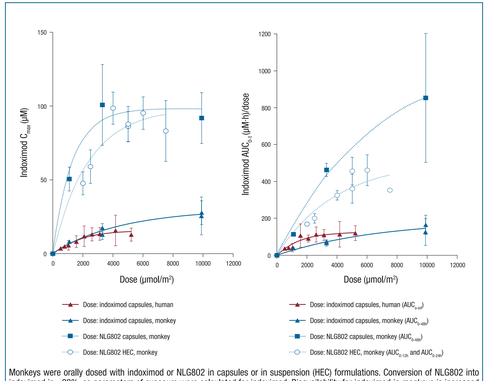
A) A B16F10 melanoma with adoptive pmel-1 T-cell transfer plus gp100 vaccination was used to estimate the dose of NLG802 needed to achieve a significant antitumor effect. NLG802 was orally dosed bid at different doses. B) PK parameters of indoximod exposure versus dose of indoximod or NLG802. PK/PD correlation suggests that the antitumor effect is observed at AUC_{0-24h} >145 μM·h and C_{max} >50 μM for indoximod.

Figure 3. Dose-dependent antitumor effect of NLG802 and PK/PD correlations in mice.



Rats were orally dosed with indoximod or NLG802 in capsules with excipients. Conversion of NLG802 into indoximod is >99% (indoximod to parent ratio: ~39000), so parameters of exposure were calculated for indoximod. Bioavailability for indoximod in rats is ~90%, which limits further increase in exposure of indoximod by NLG802.

Figure 4. PK comparison of NLG802 with indoximod in rats.



Monkeys were orally dosed with indoximod or NLG802 in capsules or in suspension (HEC) formulations. Conversion of NLG802 into indoximod is >99%, so parameters of exposure were calculated for indoximod. Bioavailability for indoximod in monkeys is increased by ~10-fold (from ~8% to ~78%) by NLG802. For comparison, dose-dependent exposure parameters for indoximod in humans after oral dosing of indoximod capsules of similar composition are shown.

Figure 5. PK of NLG802 in monkeys and indoximod in monkeys and humans.

Table 1. NLG802 Metabolization, Excretion, and Drug-Drug Interaction Risks

	% metabolite (% UV area) in hepatocytes				
Metabolite	Mouse	Rat	Dog	Monkey	Human
NLG802	0.3	0.4	1	1.3	0.9
M14 (int. met.)	–	3.8	–	7.6	1.2
Indoximod	58	74	62	71	89
M15	ND	0.4	4.8	1	3.8
M1	12	16	11	12	1.9
M8	–	–	11	–	–
M5	5.4	–	–	–	–

	Rat		Monkey	
Excretion	Urine	Feces	Urine	Feces
NLG802	<0.02%	<0.02%	<0.02%	<0.02%
Indoximod	30%-50%	~0.5%	15%	<0.2%

Target	NLG802 IC_{50} (μM)	Indoximod IC_{50} (μM)
CYP3A4: direct	5	>50
CYP3A4: TDI	7X shift	No TDI
CYP2D6	19	>50
CYPC19	46	>50
CYP 1A2, 2B6, 2C8, 2C9	>50	>50
CYP 1A2, 2B6, 3A4 induction	NO	ND
OCT1	74	>100
OCT2, OAT1, OAT3, OATP1B1, OATP1B3	>100	>100
PgP/MDCKII	27	>100
BRCP/Caco2	>100	>100

Table 2. NLG802 Toxicology and Safety Pharmacology Summary

Genetic toxicology	Species	Max dose	Result
Ames test	Bacteria	5 mg/plate	Negative
Chromosomal aberrations	Hamster (CHO)	445 μM	Negative
Bone marrow micronucleus	Rat	2000 mg/kg	Negative
Safety pharmacology	**Species**	**Doses**	**Result**
Cardiovascular	hERG/CHO	0-100 μM	EC_{50} 27 μM
	Monkey	0, 60, 120, 150 mg/kg	Negative
Respiratory	Monkey	0, 60, 120, 150 mg/kg	Negative
Neurobehavioral	Rat	0, 20, 60, 180→150 mg/kg	↓ activity and arousal at 180 mg/kg
	Monkey	0, 60, 120, 150 mg/kg	Negative
Dose repeat toxicology	**Species**	**Doses (mg/kg)**	**NOAEL (mg/kg)**	**STD10/MTD (mg/kg)**
Single-dose range finding	Rat	75, 225, 675, 2000	<2000	>2000
	Monkey	50, 150, 450, 1350	<1350	>1350
7-day, bid	Rat	100, 300→200, 900	100	200
	Monkey	75, 225→150, 675→375	150	150
28-day, bid	Rat	20, 60, 180→150	60	**150**
	Monkey	60, 120, 180→150	120	120

Table 3. Increase in Indoximod Exposure After Dosing NLG802

	Mouse				Rat			Monkey		
Dose (μmol/kg)	124	247	512	989	37	185	556	92	275	825
C_{max}	372%	384%	152%	103%	65%	119%	8%	518%	477%	190%
AUC_{0-last}	265%	121%	–2%	11%	48%	93%	19%	196%	518%	285%

Mice, rats, and monkeys received different doses of indoximod or NLG802. The average percent increase in indoximod exposure after dosing NLG802 versus indoximod is shown. Mice: suspension; rats and monkeys: capsules.

CONCLUSIONS

- **NLG802 increases indoximod exposure and plasma concentration ~3- to 6-fold in monkeys dosed with capsules of comparable formulation to that being used in clinical trials**
- **Monkeys seem to be the most appropriate species to predict the PK of NLG802 in humans**
- **NLG802 is rapidly absorbed and metabolized in vivo to indoximod in all species tested**
- **NLG802 DMPK profile and GLP toxicology studies have been carried out in rats and monkeys, which suggests a safe toxicologic profile at predicted human therapeutic doses**
- **NLG802 is a prodrug of indoximod that is predicted to increase clinical drug exposure to indoximod above the current achievable levels**

FUTURE DIRECTIONS

- An open-label, phase 1 trial utilizing a standard 3+3 dose-escalation design will be conducted to:
  1. Evaluate the safety and toxicity of NLG802 in advanced solid tumors for which no standard therapy exists
  2. Define MTD or MBAD and RP2D of NLG802 in advanced solid tumors
  3. Evaluate PK of NLG802 and its active metabolite indoximod
- Patients will receive NLG802 twice a day each day of a 28-day cycle for up to 12 months or until disease progression or other off-study criteria are met

Phase 1 cohort	Dose (mg)
1	180
2	363
3	726
4	1089
5	1452
6	1815

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