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Novel specific- and dual- tryptophan-2,3-dioxygenase (TDO) and indoleamine-2,3-dioxygenase (IDO) inhibitors for tumor immunotherapy

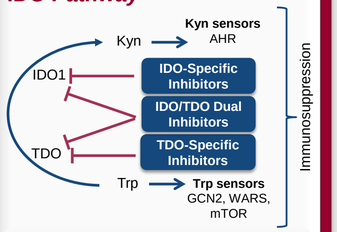
M. Mautino[1], F. Jaipuri[1], J. Waldo[1], S. Kumar[1], J. Adams[1], C. Van Allen[1], A. Marcinowicz-Flick[1], N. Vahanian[1] and C. J. Link[1]

[1] NewLink Genetics Corporation, Ames, IA

Introduction

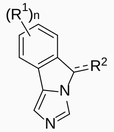
- Immuno-oncology is one of the most promising approaches in cancer and checkpoint inhibition plays a key role in immune mediated anti-tumor responses. IDO pathway plays a central role with checkpoint network that includes other key targets such as CTLA-4 and PD-1.
- Trpytophan (Trp) and kynurenine (Kyn) can act as second messengers to signal T-cell proliferation that may lead to anti-tumor responses and to promote tumor growth[1]. The IDO pathway mediates immunosuppressive effects through the metabolization of Trp to Kyn, triggering downstream signaling through GCN2[2], mTOR[3] and AHR[4], which can affect differentiation and proliferation of T cells[5], activation of regulatory T cells[6], the immunophenotype of dendritic cells in tumor draining lymph nodes[7] and promotion of tumor growth[4].
- TDO is expressed in human tumors[4,8] and could potentially be a compensatory immunosuppressive mechanism utilized by tumors in the context of IDO inhibition.
- In vitro and animal models indicate that TDO expressed by the tumor can reduce T cell proliferation and lead to immunosuppression. Previously tested proof-of-concept TDO inhibitors have demonstrated antitumor effect in animal models[8].
- Therefore, targeting Trp degradation by inhibition of IDO and/or TDO activities is a prime target for small-molecule immunomodulatory drugs in cancer.

IDO Pathway



Pharmacophore

5*H*-imidazo[5,1-*a*]isoindole



R^1= H, F, Cl, Br, OMe

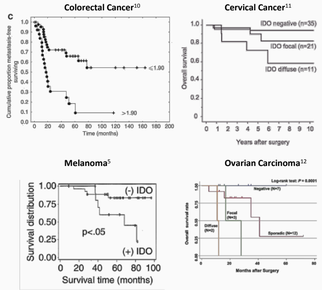
Conclusions

- We have explored a family of imidazoisoindole compounds and found potent TDO-specific , IDO-specific and dual IDO/TDO inhibitors. Compounds were classified as either TDO-specific, IDO-specific or dual IDO/TDO inhibitors based on potency in all assays and ratios of inhibitory activity.
- The identification of tumors expressing high levels of IDO or TDO may allow more selective inhibition of the Trp-regulated immunosuppressive pathways. Alternatively, the inclusion of compounds inhibiting both IDO and TDO could provide the greatest coverage to prevent tumor escape by compensatory expression of the other Trp-degrading enzyme.
- In summary, the use of dual IDO/TDO inhibitors or combinations of IDO and TDO specific inhibitors may prove to be a superior treatment alternative in immunotherapy of cancer to block immunosuppression mediated by tryptophan metabolism.

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)*** *Mautino MR – AACR 2014 #5023;* ***8)*** *Pilotte L – PNAS 2012(109)2497;* ***9)*** *Uyttenhove C – Nat. Med. 2003(9)1269;* ***10)*** *Ferdinande L - Br J Cancer 2012(106)141;* ***11)*** *Inaba T – Gynecol Oncol 2010(117)423;* ***12)*** *Okamoto – Clin Cancer Res. 2005(11)6030.*

IDO & Cancer

Tumor Type	IDO$^+$ Tumor[9]	IDO$^+$ /TDO$^-$ Cells[8]
Colorectal	10/10	1/11
Pancreatic	10/10	3/5
Glioblastoma	9/10	3/8
Ovarian	8/10	1/1
Bladder	8/10	1/3
NSCLC	9/11	0/7
Head and neck	7/11	2/11
Mesothelioma	6/10	2/7
Renal cell	5/10	1/9
Lymphoma	4/18	0/4
Melanoma	11/25	2/12
Hepatocellular	2/5	0/2
Breast	3/10	1/4
SCLC	2/10	0/6
Sarcoma	2/10	0/6



IDO is expressed in a wide variety of human tumors and tumor cell lines as well as in host APCs, which correlates with a worse clinical prognosis. Therefore, inhibition of IDO may improve survival in cancer patients with IDO-mediated immunosuppression.

IDO-Specific Inhibitors

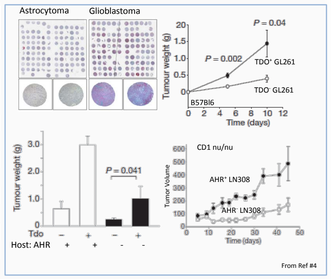
ID	hIDO IC50 (nM)	hIDO EC50 (nM)	hTDO IC50 (nM)	hTDO EC50 (μM)
NLG919	13	75	140	1.5
NLG-4311	9	30	600	2.9
NLG-4316	30	72	1730	NT
NLG-4258	16	31	590	50
NLG-4240	18	76	530	4.3
NLG-4384	6	20	120	4.6
NLG-4093	71	130	2600	52
NLG-4382	10	17	180	3.2
NLG-4571	16	190	620	2.3
NLG-4309	3	16	90	2.4
NLG-4351	41	400	840	38
NLG-4222	40	130	60	4.6
NLG-4369	50	86	450	4.4
NLG-4573	16	110	140	5.1
NLG-4540	35	70	300	2.2
NLG-4040	27	200	120	6.5
NLG-4147	31	78	260	NT
NLG-4293	57	43	400	6.5

IDO Enzymatic Assay: 70 nM hIDO, 200 μM L-Trp, 20 mM ascorbate, 20 μM methylene blue, 1000 U/mL catalase, pH6.5, 0-10 μM Test compound, 15 min at 37 °C.

IDO Cell-based Assay: 10^5 cells/well 293TRex-IDO, 100 μM L-Trp, DBZ medium, 18 h at 37 C, 0-10 μM Test compound. SN processed for Kyn, and cells for WST assay.

TDO & Cancer

Tumor Type	TDO$^+$ Tumor[8]	IDO$^-$ TDO$^+$ Cells[8]	IDO$^-$ TDO$^+$ Cells[4]
Colorectal	2/7	5/11	
Glioblastoma	2/10	1/8	4/4
Neuroblastoma	2/3		
NSCLC/SCLC	2/8	5/13	
Head and neck	1/9	1/11	
Mesothelioma	2/4	1/7	
Renal cell	1/7	4/9	
Melanoma	10/20	0/12	
Hepatocellular	7/7	0/2	
Breast	2/17	1/4	
Sarcoma	1/5	0/6	
Bladder	9/22	0/3	



TDO is expressed in a wide variety of human tumors and tumor cell lines [4,8]. Expression of TDO is evident in advanced human glioblastomas[4]. Expression of TDO in GL261 results in enhanced tumor growth[4]. This enhanced tumor growth is the consequence of an autocrine and paracrine effect caused by TDO-produced Kyn, which interacts with AHR on the tumor cell and on host immune cells[4].

TDO-Specific Inhibitors

R^2= $C_{1\text{-}6}$alkylR^A
R^A= H, OH, CN, COR^6, $COOR^6$, $CONR^6(R^7)$, $C(=N\text{-}OR^7)R^6$
$(C_{1\text{-}6}alkyl)Aryl(R^8)_n$ or $(C_{1\text{-}6}alkyl)Heteroaryl(R^6)_n$

ID	hIDO IC50 (nM)	hIDO EC50 (nM)	hTDO IC50 (nM)	hTDO EC50 (μM)
NLG-14022	34000	>50000	14	2.3
NLG-16023	8700	48000	25	7.4

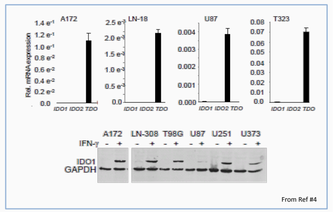
ID	hIDO IC50 (nM)	hIDO EC50 (nM)	hTDO IC50 (nM)	hTDO EC50 (μM)
NLG-3929	590	2500	41	
NLG-3980	8900	1700	44	1.7
NLG-4020	5200		38	4.6
NLG-3989	2800	8600	36	3.0
NLG-4031	3700	21000	38	9.9
NLG-3971	5200	12800	45	9.3
NLG-4120	2000	3600	41	4.2
NLG-4111	>50000	42000	88	72
NLG-4116	1800	760	100	62
NLG-4362	8800	>50000	430	
NLG-4113	13000		290	3.9

TDO Enzymatic Assay: 260 nM hTDO, 500 μM L-Trp, 10 mM ascorbate, 10 μM methylene blue, 1000 U/mL catalase, pH6.5, 0-10 μM Test compound, 45 min at 25 °C.

TDO Cell-based Assay: $5x10^4$ cells/well 293TRex-IDO, 200 μM L-Trp, DBZ medium, 18 h at 37C, 0-10 μM Test compound. SN processed for Kyn, and cells for WST assay.

IDO and TDO in Cancer

Cell Line Type	IDO$^+$ /TDO$^+$ Cells[4,8]
Colorectal	0/11
Pancreatic	1/5
Glioblastoma	1/8
Ovarian	0/1
Bladder	2/3
NSCLC	1/7
Head and neck	5/11
Mesothelioma	3/7
Renal cell	0/9
Lymphoma	0/4
Melanoma	0/12
Hepatocellular	0/2
Breast	0/4
SCLC	0/6
Sarcoma	2/6



TDO or IDO is expressed in ~ 50% of tested human tumor cell lines. Of that 50%, ~1/3 express both IDO and TDO[8]. A significant number of glioblastoma cell lines express TDO but not IDO1 or IDO2[4]. However, upon stimulation with INFg, these cells are capable of inducing IDO1[4]. Therefore, inhibition of TDO in glioblastoma, might help triggering an immune reaction to the tumor which could leads to induction of IDO1 as a compensatory immunosuppressive mechanism.

Consequently, sequential or simultaneous inhibition of both TDO and IDO1 with IDO1-specific and TDO-specific, or with dual IDO/TDO inhibitors might be a preferable course of treatment for gliomas and other tumors expressing TDO.

Dual IDO/TDO Inhibitors

ID	hIDO IC50 (nM)	hIDO EC50 (nM)	hTDO IC50 (nM)	hTDO EC50 (μM)
NLG919	13	75	140	1.5
NLG-4393	28	50	60	0.44
NLG-4173	21	75	74	0.5
NLG-3922	60	83	270	1.2
NLG-4584	15		25	15
NLG-4313	7	26	40	3.1
NLG-4389	15	23	54	4.6
NLG-4593	25		110	4.5
NLG-4104	22	9	130	2.2
NLG-4569	15	100	77	4.3
NLG-4564	21	120	110	3.9
NLG-4371	45	50	160	2.7
NLG-4575	92	130	100	13
NLG-4244	45	59	200	5.9
NLG-4131	66	39	75	
NLG-4151	46	79	110	NT

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