Q701, a selective Axl/Mer inhibitor as an immune checkpoint inhibitor

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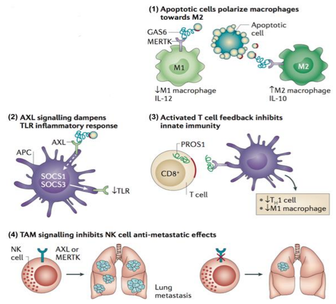
Abstract

Axl and Mer receptors are reported to have many functions including promotion of epithelial–mesenchymal transition (EMT), resistance to anti-tumor therapy and negative regulation of the innate immune response. Particular attention has been given to Axl/Mer receptor tyrosine kinase (RTK)'s roles in regulating macrophage and NK cell activation as tumor cells exploit Axl/Mer signaling pathway to down regulate anti-tumor immunity, constituting an immune checkpoint. Q701 is a highly potent, selective, orally available Axl/Mer RTK inhibitor that can relieve immune suppression through innate immunity activation leading to T cell activation. In various syngeneic mouse models, Q701 shows tumor regression activities by treatment of compound alone or in combination with other treatments, while Q701 has no direct cytotoxic activity against tested cell lines. Q701 treatment also show immune activation profile in tumor leading to T cell activation. Q701 is under IND enabling studies and a back-up program with Axl/Mer/CSF1R inhibitors is in candidate nomination stage.

Introduction



Graham, D.K.etal., Nat Rev Cancer. 14, 769-85. 2014

- Axl/Mer RTKs' physiological role is to maintain immune homeostasis after inflammatory reaction by turning off excessively activated innate immunity. The physiological function of Axl/Mer RTK is used as immune evasion pathway through activation of Axl/Mer signaling by cancer cell as well as enveloped virus.
- Axl/Mer RTK can 1) induce M2 polarization, 2) decrease of immune activation by TLR, 3) trigger negative feedback of T cell activation, and 4) suppress of NK cell activation.
- Therefore, the Axl/Mer RTK inhibition can be an effective mechanism for immunotherapy against cancer through modulation of innate immunity.

Results

Q701 is a potent and selective Axl/Mer RTK inhibitor

A In vitro activity

Enzyme Binding (Kd, nM)				Axl transfected HEK293 Cell (IC50, nM)			H1299 Cell (IC50, nM)
Axl	cMer	Tyro3	cMet	10% Serum	50% Mouse Serum	50% Human Serum	
1.6	2.9	4.5	13	3.8	56	20	1.1

B Engineered Ba/F3 cell activity

Items			Q701
Ba/F3 cell activity (IC_{50}, nM)	Axl	Ba/F3 parental BCR-None	34,200
		BCR-Axl	4.1
	Mer	Ba/F3 parental TEL-None	73,860
		TEL-Mer	2.0
	Tyro3	Ba/F3 parental TEL-None	73,860
		TEL-Tyro3	31.6

C KinomeSCAN kinase activity

Compound Name	DiscoverX Gene Symbol	Kd (nM)
Q701	AXL	0.43
	MERTK	2.2
	LOK	8.1
	MET(Y1235D)	8.2
	ABL1(H396P)-nonphosphorylated	16
	ABL1-nonphosphorylated	18
	CDK8	19
	MET	20
	FLT3	21

- Q701 has a potent Axl and Mer inhibition activities in enzyme binding assays and various cellular assays (transfected HEK293 cells, H1299 cells, or target engineered Ba/F3 cells)
- Q701 cellular activity shows less than 20 fold shift upon addition of serum
- Q701 selectivity test against 486 kinases panel identified only 7 kinases within 10 times the Kd value of Mer RTK

ADME/PK/PD properties

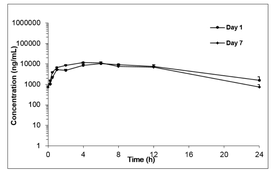
A ADME

PPB bound (%)				PPB stability (6 h, %)				Liver Microsomal stability (T_{half}, min)			
Human	Rat	Mouse	Dog	Human	Rat	Mouse	Dog	Human	Rat	Mouse	Dog
99.95	99.96	99.63	99.94	>80	>80	63	>80	144	145	145	78

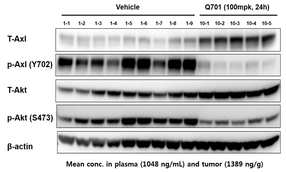
CYP Inhibition (IC50, uM)					P-gp inhibition (IC50, uM)	P-gp substrate	hERG inhibition (Patch clamp, IC50, uM)
1A2	2C19	2C9	2D6	3A4	1.23	Non-substrate	>10
>50	>50	19.4	>50	>50			

B Pharmacokinetics

PO parameters	Day 1	Day 7
Dose (mg/kg)	100	100
AUC0-last (ng•hr/mL)	137899	134267
AUC0-inf (ng•hr/mL)	151447	139136
Cmax (ng/mL)	10230	11867
Tmax (hr)	6	4
T1/2 (hr)	5.99	4.61



C Pharmacodynamics

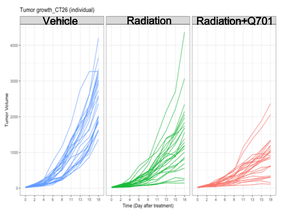


Mean conc. in plasma (1048 ng/mL) and tumor (1389 ng/g)

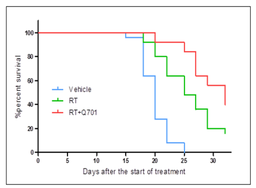
- ADME/DMPK profile of Q701 supports potential q.d. or b.i.d dosing regimen
- Q701 shows P-gp inhibition with IC50 of 1.23 uM
- Q701 inhibits Axl auto-phosphorylation up to 24h post dosing on day 7 at 100 mg/kg q.d. in H1299 xenograft model. Phospho-Axl Y702 can be considered as a PD marker
- Q701 shows 1: 1.3 compound distribution in plasma vs. tumor

Survival and tumor growth inhibition in CT26 syngeneic model

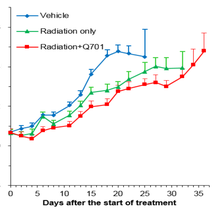
A CT26 tumor growth inhibition



B Survival rate



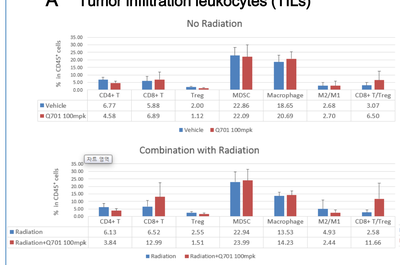
C Body weight change



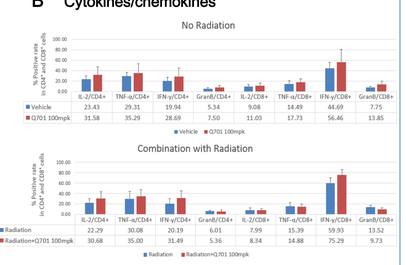
- Q701 in combination with low dose radiation shows synergistic effect in tumor growth inhibition as well as in survival prolongation (CT26 syngeneic model in Balb/c)
- Q701 shows no adverse gross finding upon daily dosing of 100 mg/kg up to 35 days

Immune profiling in CT26 syngeneic model

A Tumor infiltration leukocytes (TILs)

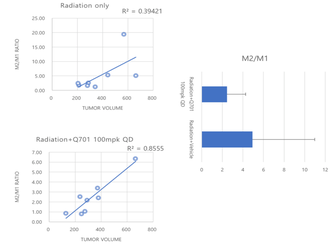


B Cytokines/chemokines

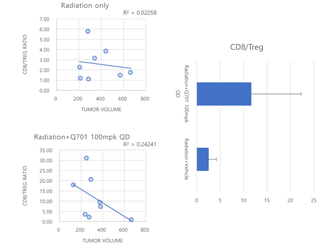


- Q701 treatment changes CD8+/Treg ratio, M2/M1 ratio and IFN-gamma contents in CD4+ and CD8+ T cells

C M2/M1 ratio



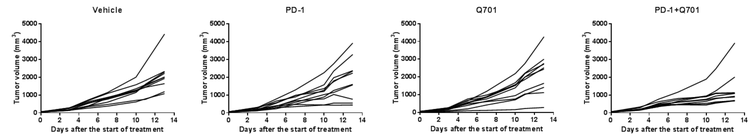
D CD8+/Treg ratio



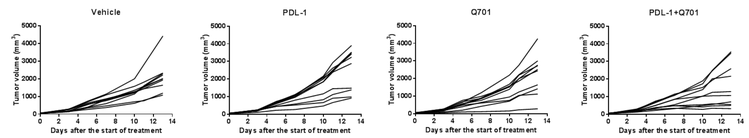
- A better correlation of tumor volume with M2/M1 ratio or CD8+/Treg ratio upon Q701 treatment indicates that Q701 may have triggered the immune profile changes leading to tumor growth inhibition

Q701 in combination with T cell immune checkpoint inhibitors

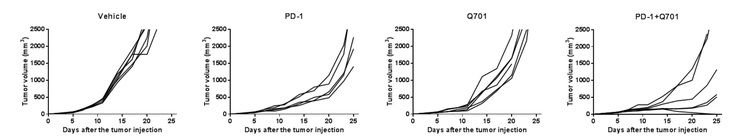
A Q701 in combination with anti-PD-1 antibody on CT26 syngeneic model



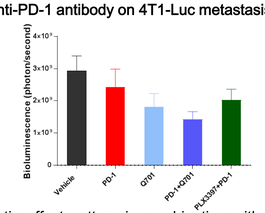
B Q701 in combination with anti-PDL-1 antibody on CT26 syngeneic model



C Q701 in combination with anti-PD-1 antibody on MC38 syngeneic model



D Q701 in combination with anti-PD-1 antibody on 4T1-Luc metastasis model



- Q701 shows additive/synergistic effect pattern in combination with anti-PD-1 or anti-PDL-1 antibody in multiple syngeneic mouse models

Conclusion

- Q701 is a highly potent, selective, orally available Axl/Mer RTK inhibitor in preclinical development
- Q701 shows no direct cytotoxicity in any of the tested cell lines (data not shown)
- In various syngeneic mouse models, Q701 shows tumor growth inhibitions as mono therapy as well as in combination with anti-PD-1 and anti-PDL-1 antibody
- Immune profiling, MOA studies and toxicology assessment are underway for IND enabling studies

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