Poster #2755

NKTR-262: Prodrug Pharmacokinetics in Mice, Rats, and Dogs

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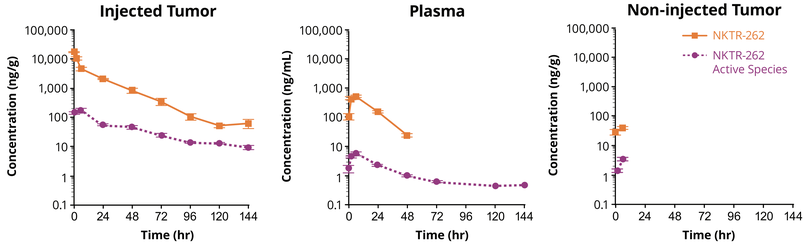
INTRODUCTION

- Agonists of TLRs (Toll-like receptors) 7/8 are currently being evaluated in clinical trials to determine their anti-tumor effect
- Efficacious plasma levels of TLR 7/8 agonists have resulted in Grade 3 or 4 adverse effects (eg, fever and lymphopenia) attributed to high systemic cytokine levels.[1,2] These results suggest that systemic exposure to a TLR 7/8 agonist should be minimized
- NKTR-262 is a small molecule agonist of TLR 7/8 designed to be retained in the tumor micro-environment. Upon intratumoral delivery, NKTR-262 provides sustained intratumoral engagement of the TLR 7/8 pathway to promote an immune stimulatory environment and tumor antigen release while reducing the peak plasma concentrations and thereby lowering the systemic cytokine induction compared to the unmodified agonist
- We present the preclinical prodrug pharmacokinetics of NKTR-262

RESULTS

Prolonged and high exposures in the injected tumor and low plasma exposures of the NKTR-262 active species after intratumoral (CT26) injection of NKTR-262

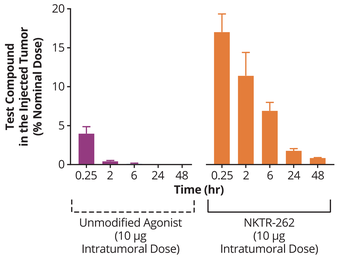


Analyte	Tissue	T_{max} (hr)	C_{max} (ng/g or ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL or hr*ng/g)	F (%)
NKTR-262	Injected tumor	NA	16,900 ± 414	176,000 ± 10,500	NA
	Plasma	6	534 ± 35.7	10,800 ± 567	26.7
	Non-injected tumor	6	40.4 ± 5.35	217 ± 14.1	NA
NKTR-262 Active Species	Injected tumor	6	176 ± 30.3	6,210 ± 388	NA
	Plasma	6	5.65 ± 0.72	190 ± 8.46	NA
	Non-injected tumor	6	3.50 ± 0.41	ND	NA

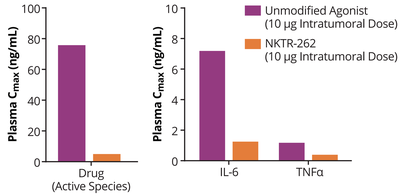
NA, not applicable; ND, not determined; T_{max}, time of observed maximum concentration; C_{max}, maximum observed concentration; $AUC_{0\text{-last}}$, area under the plasma concentration-time curve from 0 hr to the last measurable concentration; F, systemic bioavailability

RESULTS (CONTINUED)

Higher intratumoral (CT26) retention of NKTR-262 compared to the unmodified agonist



NKTR-262 exhibited lower systemic proinflammatory cytokine induction after intratumoral (CT26) injection compared to the unmodified agonist, consistent with reduced drug peak concentration in plasma



NA, not applicable; ND, not determined; T_{max}, time of observed maximum concentration; C_{max}, maximum observed concentration; $AUC_{0\text{-last}}$, area under the plasma concentration-time curve from 0 hr to the last measurable concentration; F, systemic bioavailability

- Mice were inoculated on both flanks with CT26 tumor cells (2.0 million cells per flank). When tumor volumes approached 150 mm^3, a single dose of NKTR-262 or the unmodified agonist was administered intratumorally in the right flank tumor (injected), and at predetermined time points, animals were sacrificed. Tumors (injected and non-injected) and plasma were analyzed for NKTR-262, NKTR-262 active species, unmodified agonist, IL-6, and/or TNFα
- NKTR-262 dose and concentrations are shown in NKTR-262 active species content

The prodrug concept was confirmed by the formation of NKTR-262 active species from NKTR-262 in plasma from all preclinical species and human *in vitro*

Matrix	NKTR-262 Active Species Formation Rate (nM/min)
Human plasma	0.059 ± 0.007
Dog plasma	0.066 ± 0.007
Rat plasma	0.235 ± 0.033
Mouse plasma	0.202 ± 0.028
Buffer	0.057 ± 0.005

Buffer, William's E Medium, pH 7.4

- NKTR-262 was incubated in plasma or buffer, and NKTR-262 active species concentrations were determined using a qualified LC-MS/MS method. A linear regression was performed to calculate the apparent initial release rate of the agonist from the plots of NKTR-262 active species molar concentrations over time

Oxidative metabolism of the NKTR-262 active species was mostly CYP3A4-mediated

Human Cytochrome P450 Isoform	% Contribution to Oxidative Metabolism of NKTR-262 Active Species
CYP1A2	8%
CYP3A4	92%

- The NKTR-262 active species was incubated with recombinant human cytochrome P450 isoforms

NKTR-262 exhibited low systemic clearance. Renal clearance of NKTR-262 accounted for nearly half of the total clearance. NKTR-262 showed long half-lives and low volume of distribution

NKTR-262 Pharmacokinetic Parameter	Mouse	Rat	Dog
Total plasma clearance (mL/hr/kg)	12.0	4.88	1.99
Renal clearance (mL/hr/kg)	ND	2.33	0.84
Half-life in plasma (hr)	3.77	17.3	46.0
Volume of distribution in plasma (mL/kg)	41.5	75.4	80.5

ND, not determined

- Naïve animals received a single intravenous or subcutaneous dose of NKTR-262. Plasma and urine were collected at predetermined time points and analyzed for NKTR-262 concentrations using a qualified LC-MS/MS method.
- Pharmacokinetic parameters were determined by non-compartmental analysis using Phoenix®WinNonlin® (Version 6.4)
- Renal clearance was calculated as $AMT_{0\text{-t hr}}/((AUC_{0\text{-t hr}})$*body weight), where $AMT_{0\text{-t hr}}$ is the amount excreted into urine during 0-t hr post dose, and $AUC_{0\text{-t hr}}$ is the area under the plasma concentration-time curve during 0-t hr post dose

The NKTR-262 active species exhibited low hepatic intrinsic clearance and low plasma protein binding

NKTR-262 Active Species Pharmacokinetic Parameter	Mouse	Rat	Dog	Human
Hepatic microsomal CL_{int} (µL/min/mg of protein)	20	21	8.1	11
Hepatocyte CL_{int} (µL/min/million cells)	5.1	5.7	7.8	1.6
Free fraction in plasma	0.27	0.40	0.28	0.52
Blood to plasma ratio	0.82	0.97	0.76	0.99

- Free fraction was assessed *in vitro* by Rapid Equilibrium Dialysis in plasma. The blood to plasma ratio was calculated by its concentration ratio of the reference plasma to the plasma separated from blood after spiking NKTR-262 active species to the reference plasma and blood, respectively. Apparent intrinsic clearance of NKTR-262 active species was determined in liver microsomes (0.25 mg/mL) or hepatocytes (0.5 million cells/mL)

CONCLUSIONS

- The prodrug concept of NKTR-262 was clearly demonstrated by the formation of the NKTR-262 active species in all preclinical species and human *in vitro*
- NKTR-262 exhibited superior intratumoral retention in the injected tumor when compared to the unmodified agonist after intratumoral injection
- Intratumoral administration of NKTR-262 successfully achieved pharmacologically active levels (KC_{50} of 1.34 ng/g^3) of the NKTR-262 active species in the tumor for an extended period of time while reducing unwanted systemic drug exposure. This is in agreement with observed, higher induction of proinflammatory cytokines in the treated tumor compared to plasma.[4] The tumor type (CT26 versus EMT6) had little impact on pharmacokinetics and pharmacodynamics (EMT6 data not shown)
- Our particular prodrug strategy has the potential to mitigate the risk of cytokine syndrome by reducing the plasma peak levels of pro-inflammatory cytokines compared to the unmodified agonist
- NKTR-262 was slowly eliminated via prodrug conversion and renal clearance
- The prodrug-generated NKTR-262 active species is a low clearance compound with low protein binding

REFERENCES

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