

# Anti-tumor activity and pharmacokinetics of NKTR-102, a novel PEGylated-irinotecan conjugate, in irinotecan-resistant colorectal tumors implanted in mice

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## Abstract

NKTR-102, a novel PEGylated-irinotecan conjugate, is currently in Phase I clinical development. Non-clinical studies examined the anti-tumor activity and pharmacokinetics in a mouse HT29 colorectal tumor model, which is moderately resistant to irinotecan treatment.

Intravenous administration of NKTR-102 at 40, 60 or 90 mg/kg (irinotecan-equivalent dose) on days 0, 4 and 8 to tumor-bearing mice caused marked, statistically significant ( $p < 0.0003$ ) dose-related decreases in HT29 tumor growth (relative to saline control) that persisted until termination on day 60. Intravenous irinotecan at the same doses resulted in only modest suppression of tumor growth that was short lived and not statistically different from saline control. Tumor growth delay after NKTR-102 was significantly longer than that after irinotecan at all three dose levels ( $p < 0.001$ ). Tumor regression was observed after NKTR-102 at 90 mg/kg, but not after irinotecan at any dose level.

A 40 mg/kg IV administration of NKTR-102 on days 0, 4 and 8 resulted in prolonged plasma and tumor exposure to active metabolites irinotecan and SN38 that is associated with marked suppression of tumor growth. Mean tumor SN38  $C_{max}$  of 1100 ng/g occurred on day 15, and tumor SN38 concentration was maintained above 100 ng/g observed at termination on day 60. Apparent  $t_{1/2}$  values for SN38 in plasma and tumor after the first dose of NKTR-102 were 17 and 15 days, respectively, whereas, SN38  $t_{1/2}$  values after 40 mg/kg irinotecan were in the expected range of 2-4 h. SN38 AUC values in plasma and tumor after the first dose of NKTR-102 were 531- and 366-fold greater, respectively, than those after irinotecan dosing. Subsequent doses of NKTR-102 on days 4 and 8 resulted in even greater SN38 exposure relative to irinotecan dosing, resulting from accumulation in both plasma and tumor.

In summary, NKTR-102 resulted in marked, prolonged growth suppression of HT-29 tumor, which is otherwise modestly resistant to irinotecan. Pharmacokinetic results indicate that this suppression is associated with prolonged systemic and tumor SN38 exposure resulting from slow disposition and metabolism of NKTR-102.

## Background

NKTR-102 is a PEGylated conjugate of irinotecan, an antineoplastic agent of the topoisomerase I inhibitor class that is widely used to treat colorectal cancer and other solid tumors. NKTR-102 was created using Nektar's small molecule PEGylation technology and is currently in Phase I clinical development.

Adding a PEG moiety to anti-tumor drugs such as irinotecan is expected to lead to a decrease in drug clearance and an increase in exposure to active drug (see ECCO 14 poster 727 for details), causing greater inhibition of tumor growth.

## Objectives

- To evaluate the comparative anti-tumor activity of NKTR-102 and irinotecan in a mouse HT29 colorectal tumor model, which is moderately resistant to irinotecan.
- To determine if inhibition of tumor growth can be explained by a decrease in drug clearance and a greater accumulation of the active metabolite SN38 in colon tumor tissue.

## Materials and Methods

Two studies of NKTR-102 were conducted using the mouse HT29 colorectal tumor model.

### Tumor Growth Delay/Inhibition

Female athymic (Ncr:Nu) mice were implanted subcutaneously with HT29 tumor fragments and the tumors were allowed to reach a weight range of 135 to 184 mg. Each group of mice (n=10) was dosed intravenously every fourth day for a total of three doses of NKTR-102 or irinotecan at 40, 60, or 90 mg/kg (irinotecan-equivalent doses). The control group received normal saline. The animals were weighed and the tumors measured twice weekly after administration of the first drug injection.

### Tumor Growth PK/PD

Female athymic (Ncr:Nu) mice were implanted with HT29 tumor fragments as described above. NKTR-102 or irinotecan was administered intravenously every 4 days for three doses at a dose of 40 mg/kg (irinotecan-equivalent dose). PK sampling of plasma and tumor was scheduled at preselected time-points up to 60 days for both groups. At each time-point, four mice were sacrificed, tumors measured, and blood and tumor samples were collected for analysis via LC/MS/MS.

## Results

### Growth Delay/Inhibition

- NKTR-102 substantially suppressed tumor growth compared to controls in a statistically-significant ( $p < 0.0003$ ) dose-related manner (Figure 1). Irinotecan-treated groups did not show a statistically significant decrease in tumor growth compared to the control group.
- Tumor growth delay (T-C value) was significantly longer at all doses of NKTR-102 compared to irinotecan at all three doses tested ( $p < 0.001$ , Table 1). At 90 mg/kg, suppression persisted until termination on day 60.
- Tumor regression was observed after NKTR-102 at 90 mg/kg, but not after irinotecan at any dose level (Table 1).
- All doses of NKTR-102 and irinotecan were well tolerated with negligible loss in body weight.

Figure 1. Growth delay curves following treatment with NKTR-102 (left) and irinotecan (right).

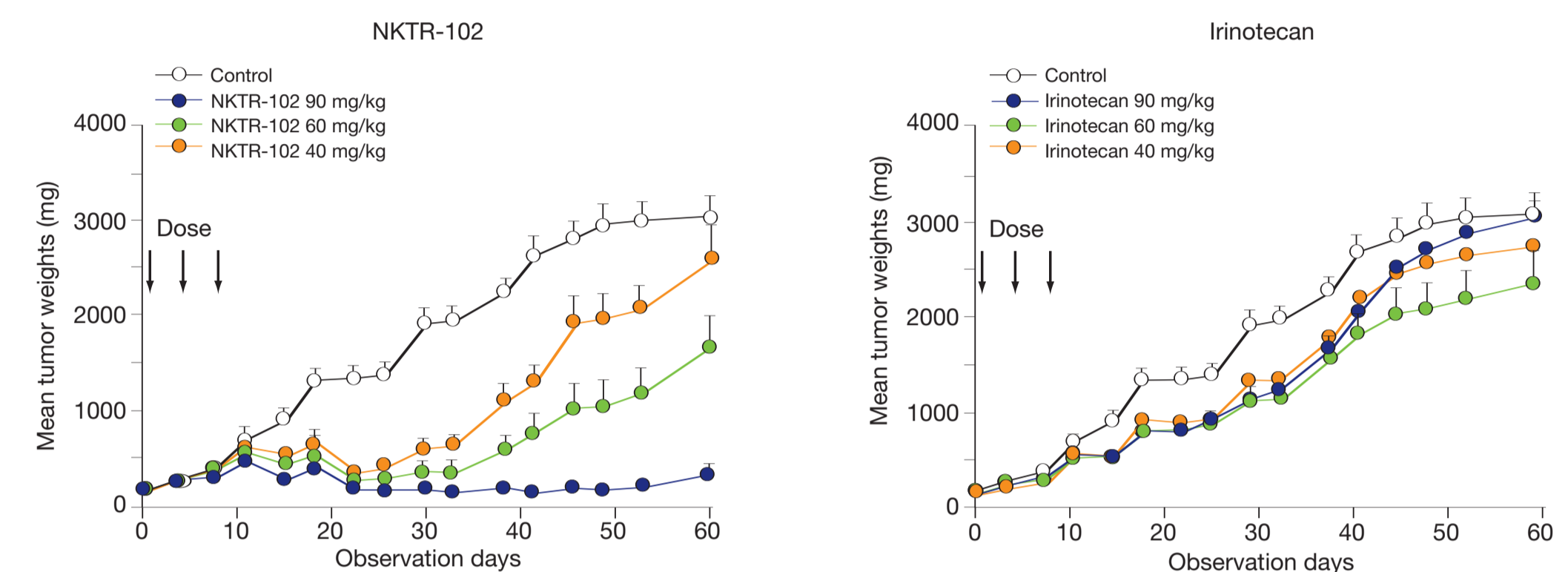


Table 1. Summary of tumor growth parameters for HT29 tumor-bearing mice following treatment with NKTR-102 or irinotecan.

Test compound	Dose (mg/kg)	Tumor regressions <sup>a</sup>		Median duration of regression <sup>b</sup> (days)	Days to 2 times tumor weight <sup>c</sup>	T-C <sup>d</sup> (days)
		Partial	Complete			
Control	0	0/5	0/5	NA	11.8	NA
NKTR-102	90	1/10	3/10	12.4	>60	>48.2
NKTR-102	60	0/9	0/9	NA	38.1	26.3
NKTR-102	40	0/9	0/9	NA	32.7	20.9
Irinotecan	90	0/6	0/6	NA	12.1	0.3
Irinotecan	60	0/4	0/4	NA	16.1	4.3
Irinotecan	40	0/3	0/3	NA	13.4	1.6

<sup>a</sup> Tumor regression: smallest tumor weight after the beginning of treatment relative to that observed on the first day of treatment. Partial: <50% of weight observed on day 1; Complete: unpalpable.  
<sup>b</sup> Interval during which partial or complete tumor regression was observed.  
<sup>c</sup> Median number of days for tumor to double in weight from the original weight.  
<sup>d</sup> Difference in the median of time for tumors to gain two times original weight for the group minus that for the control group.  
 NA = Not applicable.

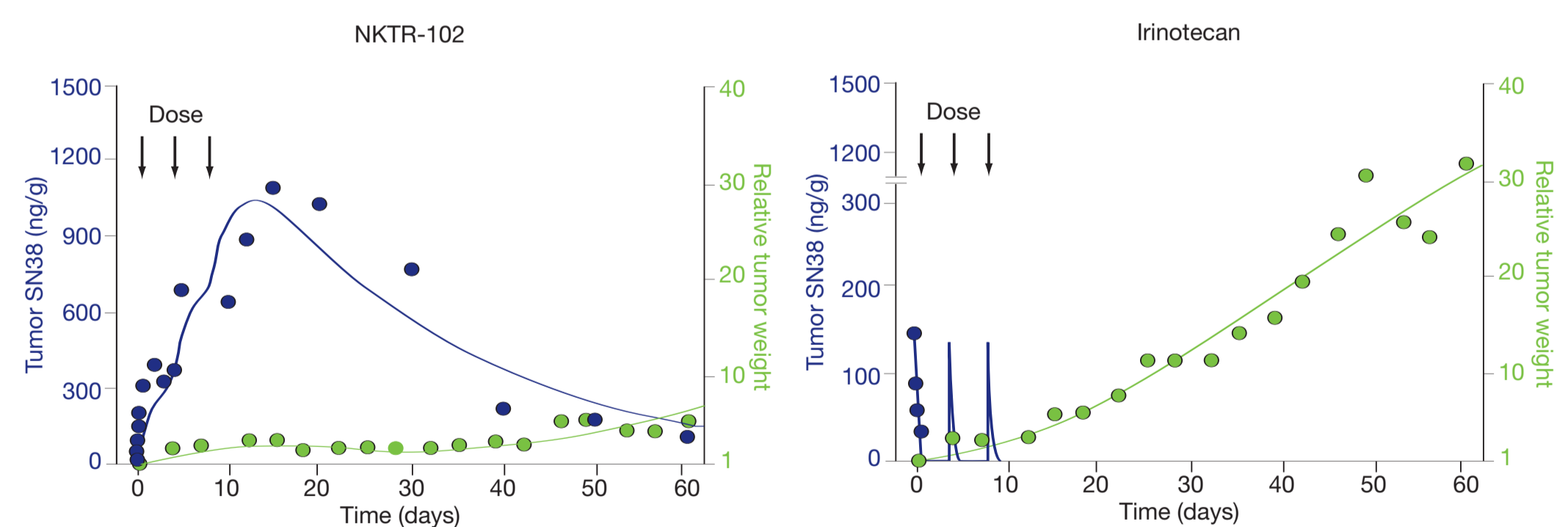
### Pharmacokinetics and Pharmacodynamics

- SN38 concentrations resulting from NKTR-102 administration declined at a much slower rate (terminal  $t_{1/2}$ ) than with irinotecan administration yielding greater exposure values (AUC, Table 2).
- Tumor suppression is associated with prolonged systemic and tumor SN38 exposure secondary to extended disposition and metabolism of NKTR-102 (Figure 2).

Table 2. PK parameters of SN38 in plasma and tumor following NKTR-102 or irinotecan administration.

SN38 PK parameters	40 mg/kg NKTR-102 dosing		40 mg/kg irinotecan dosing		NKTR-102 / irinotecan ratio	
	Plasma	Tumor	Plasma	Tumor	Plasma	Tumor
Terminal $t_{1/2}$	17 days	15 days	2 hours	4 hours	240	90
Day 1 AUC ( $\mu\text{g/ml}$ [or g] days)	5.8	11.7	0.01	0.03	531	366

Figure 2. PK/PD relationships following 40 mg/kg of NKTR-102 (left) or irinotecan (right) administration.



## Conclusions

- NKTR-102 substantially suppressed tumor growth in a statistically significant dose-related manner, while irinotecan-treated groups did not show a statistically significant decrease in tumor growth relative to control. Tumor regression was observed after NKTR-102 at 90 mg/kg, but not after irinotecan at any dose level.
- NKTR-102 inhibited tumor growth in a dose-dependent manner with 94%, 64%, and 37% tumor growth inhibition at 90 mg/kg, 60 mg/kg, and 40 mg/kg doses, respectively, on day 50.
- NKTR-102 administration to mice results in a 90-fold increase in tumor SN38 half-life (15 days versus 4 h) and a >300-fold increase in tumor SN38 AUC, respectively, compared to irinotecan at equivalent doses.
- Using Nektar's small molecule PEGylation technology, NKTR-102 was created to improve the time-concentration profile of irinotecan, resulting in substantially improved anti-tumor activity versus irinotecan in a mouse HT29 colorectal tumor model. The safety and tolerability of the molecule are being evaluated in clinical trials.