Intravenous infusions of TriN 2755, a new alkylating agent, inhibit the growth of human MDA-MB-231 breast tumour in Nude rats

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Introduction

The new cytotoxic anti-neoplastic agent TriN 2755, containing a triazene unit with alkylating properties, exhibited a potent antitumour activity in a large panel of human xenograft tumour models in mice when dosed by IV *bolus* injection. In rat, dog and mini pig toxicity studies, TriN 2755 demonstrated a favourable safety profile. Here, we present efficacy data of TriN 2755 intravenously injected in the MDA-MB-231 tumour bearing Nude rat model.

Material and Methods

- **Test substances**
  - TriN 2755 prepared in saline
  - Docetaxel prepared in Ethanol/Polysorbate 80/saline (5/5/90)
  - CPT-11 prepared in saline
- **Tumour cell line : human MDA-MB-231 breast adenocarcinoma**
- **Animals : Nude rats (Harlan SD. Inc.Indianapolis)**
- **Drug administration:**
  - IV *bolus* and IV infusion during 4 hours for TriN 2755
  - IV *bolus* for docetaxel and CPT-11
  - IV *bolus* through the tail vein
  - IV infusion *via* heparin-coated catheter implanted in the femoral vein and a perfusor
- **Tumour induction and randomisation**
  - SC inoculation of $2x10^7$ MDA-MB-231 cancer cells in matrigel to Nude rats
  - Randomization of rats based on tumour volumes at 400-600 mm^3

Number of rats	Treatment	Dose (mg/kg/adm)	Administration route	Treatment schedule
3	Control	-	-	-
3	TriN 2755	160	IV *bolus*	TWx4
3	TriN 2755	240	IV *bolus*	TWx4
3	TriN 2755	360	IV *bolus*	TWx4
8	TriN 2755 vehicle	-	4H-IV infusion	TWx4
8	TriN 2755	30	4H-IV infusion	TWx4
8	TriN 2755	80	4H-IV infusion	TWx4
8	TriN 2755	240	4H-IV infusion	TWx4

Number of rats	Treatment	Dose (mg/kg/adm)	Administration route	Treatment schedule
3	Control	-	-	-
3	TriN 2755	240	4H-IV infusion	TWx4
3	CPT-11	40	IV *bolus*	Q7Dx4
3	TriN 2755 + CPT-11	240 + 40	4H-IV infusion / IV *bolus*	TWx4 / Q7Dx4
3	TriN 2755 + docetaxel	240 + 2.5	4H-IV infusion / IV *bolus*	TWx4 / Q7Dx4

Tw: Twice weekly Q7D: Once weekly

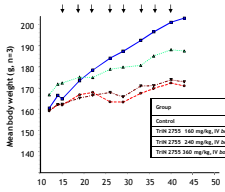
- **Toxicological and antitumour profiles**
  - Daily monitoring of rat survival
  - Managment of the study (dosing, collection, measurements…), raw data, lethality, behaviour and autopsy were recorded and analysed using Vivo Manager software (Biosystems, Dijon) [1] and[2]
  - Twice a week monitoring of rat body weight and tumour volumes
  - Rat hematological follow-up one day after the last treatment with TriN 2755 (single agent)
  - Monitoring of white blood cells (WBC)/red blood cells (RBC)/platelets
  - Histology of liver, kidney and bone marrow one day after the last treatment with TriN 2755 (single agent)
  - Clonogenicity assay issued from tumours collected 4 days after the last treatment with TriN 2755 (combination agents)

(1). Principe d'éthique de l'expérimentation animale. Directive n° 86/609 CEE du 24 Nov. 1986, Décrêt n° 87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.

(2). WORKMAN P. et al., UKCCCR guideline. Br. J. Cancer, 77: 1-10, 1998.

Results (single agent)

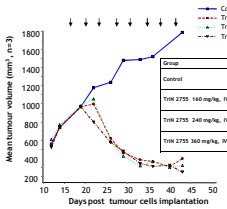
No body weight loss of MDA-MB-231 tumour bearing Nude rats treated with TriN 2755 administered by IV *bolus* injection was observed during the course of the study.



Group	No rats at D15	MBWC (D15-D40, g)	SD	MBWC (D15-D40, %)	SD
Control	3	36.26	2.10	22.02	1.50
TriN 2755 160 mg/kg, IV *bolus*: TWx4	3	17.22	3.70	11.14	3.67
TriN 2755 240 mg/kg, IV *bolus*: TWx4	3	15.75	8.64	9.35	5.75
TriN 2755 360 mg/kg, IV *bolus*: TWx4	3	11.54	7.50	7.28	4.88

MBWC: Mean Body weight change between D15-D40
MBWC (%): Mean Body weight change between D15-D40 compared to the mean body weight at D15
SD: Standard Deviation

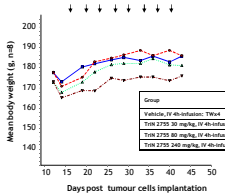
TriN 2755 displayed a marked antitumour activity in the MDA-MB-231 tumour bearing Nude rats model when administered by IV *bolus* injection.



Group	Parameters	D43
Control	Median tumour volume (mm^3)	1863.6
	T/C%	100
TriN 2755 160 mg/kg, IV *bolus*: TWx4	Median tumour volume (mm^3)	395.7
	T/C%	21
TriN 2755 240 mg/kg, IV *bolus*: TWx4	Median tumour volume (mm^3)	254.6
	T/C%	19
TriN 2755 360 mg/kg, IV *bolus*: TWx4	Median tumour volume (mm^3)	219.2
	T/C%	12

T/C%: Ratio of the median tumor volumes of treated group (T) versus control group (C)

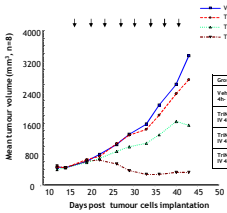
No body weight loss of MDA-MB-231 tumour bearing Nude rats treated with TriN 2755 administered by IV infusion was observed during the course of the study.



Group	No rats at D14	MBWC (D14-D43, g)	SD	MBWC (D14-D43, %)	SD
Vehicle, IV 4h-infusion: TWx4	8	14.14	11.66	8.72	7.27
TriN 2755 30 mg/kg, IV 4h-infusion: TWx4	8	15.07	8.92	9.02	5.50
TriN 2755 80 mg/kg, IV 4h-infusion: TWx4	8	12.26	5.28	7.35	3.18
TriN 2755 240 mg/kg, IV 4h-infusion: TWx4	8	10.76	9.34	6.68	6.07

MBWC: Mean Body weight change between D14-D43
MBWC (%): Mean Body weight change between D14-D43 compared to the mean body weight at D14
SD: Standard Deviation

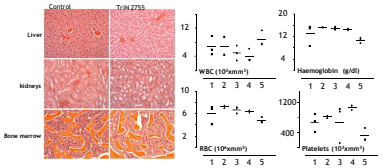
TriN 2755 displayed a marked and dose-dependant antitumour activity in the MDA-MB-231 tumour bearing Nude rats model when administered by IV infusion.



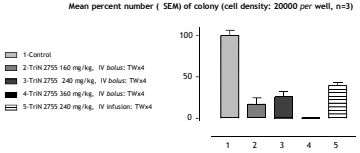
Group	Parameters	D43
Vehicle, IV 4h- infusion: TWx4	Median tumour volume (mm^3)	4157.9
	T/C%	100
TriN 2755 30 mg/kg, IV 4h-infusion: TWx4	Median tumour volume (mm^3)	2643.2
	T/C%	64
TriN 2755 80 mg/kg, IV 4h-infusion: TWx4	Median tumour volume (mm^3)	1524.7
	T/C%	37
TriN 2755 240 mg/kg, IV 4h-infusion: TWx4	Median tumour volume (mm^3)	328.1
	T/C%	7

T/C%: Ratio of the median tumor volumes of treated group (T) versus vehicle treated group (C)

No histology associated toxicity was observed in the liver, kidneys and bone marrow of rats treated with TriN 2755 administered by IV infusion at the highest dose. No white blood cell, haemoglobin and red blood cell level changes was observed for TriN 2755 treated rats (IV *bolus* and infusion) when compared to control rats. A decrease in platelets level was observed for rats treated with TriN 2755 at 240 mg/kg administered by IV infusion.

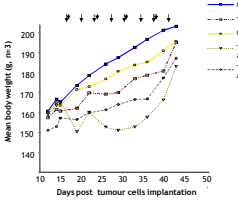


The clonogenic assay showed that the number of colonies issued from TriN 2755 MDA-MB-231 treated tumors (both IV *bolus* and infusion) was decreased compared to tumours from control rats.



Results (combination)

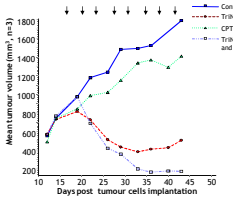
No body weight loss was observed for MDA-MB-231 tumour bearing Nude rats treated with TriN 2755 by IV infusion in combination with CPT-11. Compared to control rats, A slight and transient body weight loss was observed for Nude rats treated with TriN 2755 by IV infusion in combination with docetaxel when compared to control rats.



Group	No rats at D15	MBWC (D15-D40, g)	SD	MBWC (D15-D40, %)	SD
Control	3	36.26	2.10	22.02	1.50
TriN 2755 240 mg/kg, IV infusion: TWx4	3	20.10	5.72	12.53	3.55
CPT-11 40 mg/kg, IV *bolus*: Q7Dx4	3	27.40	5.12	17.07	4.79
TriN 2755 240 mg/kg, IV infusion: TWx4 and docetaxel 2.5 mg/kg, IV *bolus*: Q7Dx4	3	0.68	6.90	0.55	4.20
TriN 2755 240 mg/kg, IV infusion: TWx4 and CPT-11 40 mg/kg, IV *bolus*: Q7Dx4	3	20.07	7.27	12.73	4.36

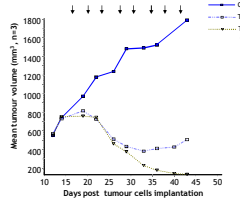
MBWC: Mean Body weight change between D15-D40
MBWC (%): Mean Body weight change between D15-D40 compared to the mean body weight at D15
SD: Standard Deviation

The antitumor activity of TriN 2755 combined with docetaxel or CPT-11 was superior to that of each drug alone.



Group	Parameters	D43
Control	Median tumour volume (mm^3)	1863.6
	T/C%	100
TriN 2755 240 mg/kg, IV infusion: TWx4	Median tumour volume (mm^3)	454.4
	T/C%	24
CPT-11 40 mg/kg, IV bolus: Q7Dx4	Median tumour volume (mm^3)	1365.2
	T/C%	73
TriN 2755 240 mg/kg, IV infusion: TWx4 and CPT-11 40 mg/kg, IV bolus: Q7Dx4	Median tumour volume (mm^3)	176.2
	T/C%	9

T/C%: Ratio of the median tumor volumes of treated group (T) versus control group (C)

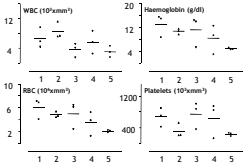


Group	Parameters	D43
Control	Median tumour volume (mm^3)	1863.6
	T/C%	100
TriN 2755 240 mg/kg, IV infusion: TWx4	Median tumour volume (mm^3)	454.4
	T/C%	24
TriN 2755 240 mg/kg, IV infusion: TWx4 and docetaxel 2.5 mg/kg, IV bolus: Q7Dx4	Median tumour volume (mm^3)	131.3
	T/C%	7

T/C%: Ratio of the median tumor volumes of treated group (T) versus control group (C)

A decrease of white blood cell, haemoglobin, red blood cell and platelet levels was observed for rats treated with TriN 2755 at 240 mg/kg/inf in combination with CPT-11.

1-Control
2-TriN 2755 240 mg/kg, IV infusion: TWx4
3-CPT-11 40 mg/kg, IV *bolus*: Q7Dx4
4-TriN 2755 240 mg/kg, IV infusion: TWx4 and docetaxel 2.5 mg/kg, IV *bolus*: Q7Dx4
5-TriN 2755 240 mg/kg, IV infusion: TWx4 and CPT-11 40 mg/kg, IV *bolus*: Q7Dx4



Conclusions

- No significant adverse related side toxicity of TriN 2755 was observed in tumour bearing Nude rats after IV *bolus* and IV infusion,
- TriN 2755 injected by IV *bolus* and IV infusion displayed a marked antitumour activity in MDA-MB-231 breast tumour in Nude rats,
- The antitumour activity of TriN 2755 combined with docetaxel and CPT-11 was higher than the one of each drug alone,
- These data support the clinical development of TriN 2755,
- TriN 2755 is currently under evaluation in phase I clinical trials for the treatment of solid tumours.