Antitumor activity study of Vinorelbine against a human PC-3 prostate tumor xenografted in *Nude* rats.

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1- Oncodesign, Dijon, France; 2- Laboratoires Pierre Fabre Oncologie, Boulogne, France.

ABSTRACT # 1329

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Vinorelbine (VRL) is a semi-synthetic Nor-5' anhydrovinblastine which inhibits mitotic microtubule polymerization. The aim of this study was to investigate the antitumor activity of VRL intravenously (IV) weekly injected (Q7Dx4) in *Nude* rats bearing subcutaneous (SC), metastatic and orthotopic PC-3 or PC-3 M human prostate tumors.

The maximal tolerated dose (MTD) of VRL IV Q7 Dx4 injected in tumor-bearing *Nude* rats was between 2.0 and 2.5 mg base/kg/injection (inj), depending on the location of the tumor growth.

In the SC PC-3 tumor model (107 PC-3 cells SC injected at D0 into whole body irradiated rats), a significant loss of body weight was observed for rats treated with both VRL (at 2.0 and 2.5 mg base/kg/inj) and Taxotere (TXT at the MTD: Q7 Dx4 IV at 5.0 mg base/kg/inj) compared to the vehicle group. The T/C% parameters provided evidence of significant antitumor activity for VRL and TXT compared to the vehicle group (T/C% of 3.0% at D54 for both groups). 100% of PC-3 tumors were cured by VRL treatment, while only 20% of them were cured by TXT treatment at D119.

In the disseminated PC-3 metastatic model, irradiated *Nude* rats were intracardially injected with 5 106 PC-3 cells at D0. The autopsies of rats from the vehicle group revealed the presence of bone metastasis in femora, tibia, ribs and lymph nodes in 91.3% of the rats. A significant loss of body weight was observed for rats treated with VRL (-12.4 vs +3.3%) compared to the vehicle group. The mean survival time of rats from the vehicle and VRL-treated groups were 28.7 ± 1.6 and 51.8 ± 26.7 days, respectively. The T/C% parameters also provided evidence of an increase in survival of VRL-treated rats compared to the vehicle treated rats (T/C% of 150.0%).

In the orthotopic model, irradiated rats were grafted in the prostate with a tumor fragment obtained from SC tumors induced by inoculation of PC-3M cells, a metastatic variant of PC-3 cells (generous gift of I. Fidler) in *Nude* rat.

The autopsies of rats from the vehicle group revealed the presence of PC3-M tumor cells disseminated via the lymph nodes in the peritoneal cavity, epiploon, spleen and diaphragm (PCR detection). No significant loss of body weight was observed for rats treated with VRL. The mean survival time of vehicle and VRL treated groups were 23.2 ± 7.0 and 38.0 ± 20.9 days, respectively. The mean weight of the primary tumor from the treated group was significantly lower than in control group and one *Nude* rat from the treated group was still alive at D79.

VRL displayed a significant antitumor activity against SC, disseminated and orthotopic PC-3 tumors growing in *Nude* rats.

INTRODUCTION

Vinorelbine (VRL, Navelbine®) is a semisynthetic Nor-5'-anhydrovinblastine, modified on the catharantine ring.

The main experimental toxicity of VRL is a reversible leucopenia. Navelbine® is currently used in clinic for the treatment of Non Small Cell Lung Cancer and metastatic Breast Cancer.

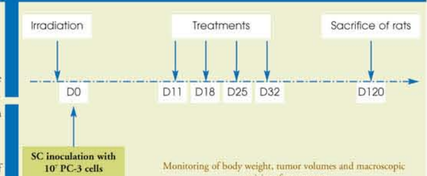
STUDY AIMS

- To study the antitumor activity of VRL against subcutaneous (SC) human PC-3 androgen-independent prostatic carcinoma xenografted in *Nude* rats,
- To study the antitumor activity of VRL against human PC-3 androgen-independent prostate carcinoma disseminated in *Nude* rats,
- To study the antitumor activity of VRL against a metastatic variant of PC-3 human androgen-independent prostate tumors xenografted orthotopically in *Nude* rats.

METHODOLOGY

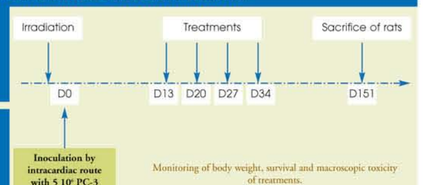
SC PC-3 MODEL: EXPERIMENTAL DESIGN AND TREATMENTS

- Test substance: VRL
- Reference article: TXT
- Animals: Male *Nude* rats, 4-5 weeks-old,
- 24 hours after a whole body irradiation at 7.0 Grays, the *Nude* rats were SC inoculated with 10^7 PC-3 cells at D0,
- Treatment schedule: Four weekly repeated IV injections of VRL or TXT with 7 days interval (Q7 DX4) (treatment start at D11),
- Doses of VRL and TXT at 2.5 and 5.0 mg base/kg/inj., respectively.
- Monitoring of body weight, tumor volumes and macroscopic toxicity of treatments.



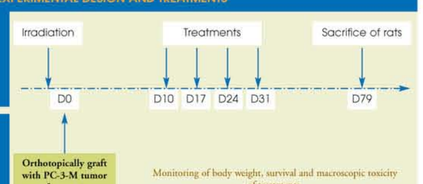
DISSEMINATED PC-3 METASTASIS MODEL: EXPERIMENTAL DESIGN AND TREATMENTS

- Test substance: VRL
- Animals: Male *Nude* rats, 4-5 weeks-old,
- 24 hours after a whole body irradiation at 7.0 Grays, the *Nude* rats were inoculated by intracardiac route with $5 \cdot 10^6$ PC-3 cells at D0,
- Treatment schedule: Four weekly repeated IV injections of VRL with 7 days interval (Q7 DX4) (treatment start at D13),
- Doses of VRL at 2.5 mg base/kg/inj.,
- Monitoring of body weight, survival and macroscopic toxicity of treatments.



ORTHOTOPIC PC-3 TUMOR MODEL: EXPERIMENTAL DESIGN AND TREATMENTS

- Test substance: VRL
- Animals: Male *Nude* rats, 4-5 weeks-old,
- 24 hours after a whole body irradiation at 7.0 Grays, the *Nude* rats were orthopically (OT) graft with PC3-M tumor fragments at D0,
- Treatment schedule: Four weekly repeated IV injections of VRL with 7 days interval (Q7 DX4) (treatment start at D 10)
- Doses of VRL at 2.0 - 2.5 mg base/kg/inj.,
- Monitoring of body weight, survival and macroscopic toxicity of treatments.

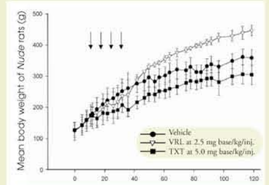


RESULTS SC PC-3 MODEL

TOXICITY

Groups	Treatment	Dose VRL or TXT (mg base/kg/inj)	No. rats	No. rat dead by toxicity of treatments	Surviving rat (%)	MBWC ± SD (g)	MBWC (%)	Mean DT ± SD (Days)
1	Vehicle	0.0	10	0	100	+19.99 ± 11.50	+11.50 ± 2.60	12.64 ± 3.86
2	VRL	2.5	10	7	30	+4.41 ± 16.84	+1.49 ± 9.84	ND
3	TXT	5.0	10	0	100	-10.34 ± 11.13	-5.60 ± 5.57	ND

- 7 of 10 rats (70%) from group 2 were found dead during the treatment period (D13-D32),
- A significant loss of body weight of rats from groups 2 and 3 was observed when compared to group 1 between D11-D15.

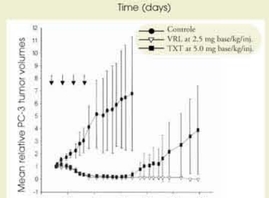


ANTITUMOR ACTIVITY

Groups	Treatm.	Days	Time (Days) 11	12	15	19	22	26	29	32	36	42	47	50	54
1	Vehicle	Median tumor volumes (mm³)	205.64	233.23	328.22	330.23	408.31	470.78	512.70	612.61	805.87	1016.63	1019.45	995.57	1067.31
2	VRL	Median tumor volumes (mm³)	204.98	244.87	200.83	236.65	192.96	157.87	91.04	90.08	54.03	32.00	32.00	32.00	32.00
		T/C %	99.68	104.99	61.19	71.66	47.26	33.53	17.76	14.70	6.70	3.15	3.14	3.21	3.00
3	TXT	Median tumor volumes (mm³)	202.35	214.90	227.99	217.70	186.16	132.26	88.16	65.65	60.05	52.20	47.84	42.21	32.00
		T/C %	98.39	92.14	69.46	65.93	45.59	28.09	17.19	10.72	7.45	5.13	4.69	4.24	3.00

Significant antitumor activity when T/C% < 42%

- At sacrifice (D119), 100% of PC-3 tumors from surviving rats were cured by VRL treatments.



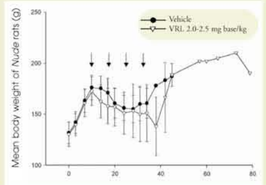
RESULTS ORTHOTOPIC PC-3 TUMOR MODEL

TOXICITY

Groups	Treatment	Adm. route	Dose VRL (mg base/kg/inj)	No. rat	No rat dead by toxicity of treatments	No. surviving rat at D79	Take-rate	MBWC ± SD(g)	MBWC (%)
1	Vehicle	IV	0.0	12	0	0	100	-22.09±9.78	-11.32±5.14
2	VRL	IV	2.0-2.5	13*	1*	1	NA	-22.94±16.09	-13.11±9.49

*: *One rats was dead just after injection of VRL*

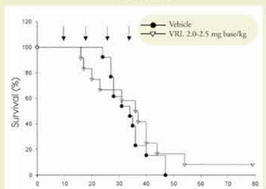
- 1 of 13 rats (7.7%) from group 2 were found dead after the second treatment (D20).
- No significant loss of body weight of rats from groups 2 was observed when compared to group 1 between D10-D24.



ANTITUMOR ACTIVITY

Groups	Treatment	Survival rate at D79 (%)	Mean survival time ± SD (days)	Median survival time (days)	ILS (%)	T/C (%)	No rat autopsied at the death	No rat with metastasis (%)	Mean primary tumor weight at the death ± SD (g)
1	Vehicle	0	33.2 ± 7.0	33.0	NA	NA	6	100% (6/6)	17.7 ± 7.4
2	VRL	8.3	36.9 ± 18.0	37.5	13.6	113.6	7	71% (5/7)	5.4 ± 3.9

- The autopsies of all dead rats from group 1 revealed the presence of PC3-M metastasis in epiploon, iliac nodes, spleen and diaphragm,
- Moreover, their autopsy revealed an hypertrophy of kidneys and the presence of ascitic fluid in the pleural cavity,
- The autopsies of 71% of dead rats from group 2 revealed the presence of PC3-M metastasis in the sites previously described for group 1,
- The mean primary tumor weight at the death of rats from group 2 were significantly smaller than for group 1,
- One rat (8.3%) from group 2 was still alive at D79.



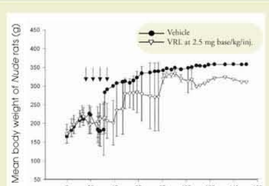
RESULTS: DISSEMINATED PC-3 METASTASIS MODEL

TOXICITY

Groups	Treatment	Adm. route	Dose VRL (mg base/kg/inj)	No. rat	No. surviving rat at D151	Take-rate (%)	MBWC ± SD (g)	MBWC (%)
1	Vehicle	IV	0.0	12*	1	92	+7.4 ± 9.6	+3.3 ± 4.3
2	VRL	IV	2.5	12§	1	NA	-27.8 ± 8.6	-12.4 ± 3.7

*: *1 rat was dead after IC injection of cells*
§: *2 rats were dead during the second treatment*

- 2 of 12 rats (16%) from group 2 were found dead after the second treatment (D20),
- A significant loss of body weight of rats from groups 2 was observed when compared to group 1 between D13-D19.

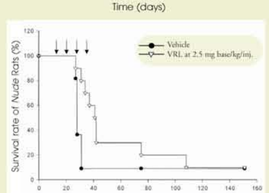


Antitumor activity

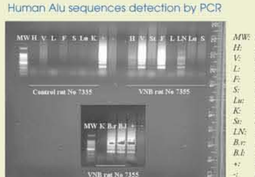
Groups	Treatment	Survival rate at D151 (%)	Survival rate at D151subtracted by "no-take" (%)	Mean survival time ± SD (days)	Median survival time (days)	ILS(%)	T/C(%)
1	Vehicle	9.1	0.0	28.7 ± 1.6	28.0	NA	NA
2	VRL	10.0	0.9	51.8 ± 26.7	42.0	50.0	150.0

Significant antitumor activity when T/C% > 125%

- The autopsies of dead rats revealed the presence of bone metastasis in femora, tibia, intercostal bone, adrenal glands, and tumors in lymph nodes, mesenteric nodes and axillary nodes,
- The surviving rats in groups 1 and 2 were sacrificed at D151,
- Their autopsy revealed the presence of macroscopic metastasis in rat from group 2 and not from group 1,
- The heart, spinal cord, liver, femora, spleen, lungs, kidneys, stomach , lymph nodes, bladder were collected from rats in order to detect the human Alu sequences by PCR.



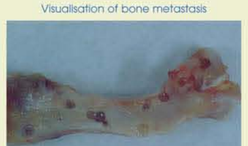
Human Alu sequences detection by PCR



MW: Molecular weight - 100 pb ladder
H: Heart
V: Spinal cord
L: Liver
F: Femur
S: Spleen
Lu: Lungs
K: Kidneys
St: Stomach
LN: Lymph node
Br: Right bladder
Bl: Left bladder
+: Positive control (U937 human cells)
-: Negative control (mouse tail)

The human Alu sequence were found in spinal cord, femur, lymph node and bladder of rat treated with VRL and not in control rat.

Visualisation of bone metastasis



CONCLUSIONS

- **VRL displayed a significant antitumor activity on:**
  - **SC PC-3 tumors xenografted in *Nude* rats, but the used schedule was not well tolerated.**
  - **PC-3 metastasis disseminated in *Nude* rats,**
  - **OT PC-3 tumor xenografted in *Nude* rats.**
- **The *Nude* rat model is particularly adapted to study the antitumor activity of drugs in preclinical development. Its size allows various injection routes for cells (SC, IC, OT, …), for drugs (IV, SC, PO, IP, continuous infusion, …) and multiple and repetitive sampling (blood, urine, faeces, …).**

AACR - Graphics by NL - PFO Unit: 29/2002