Disseminated human hematological malignancies xenografted in athymic mice dedicated to targeted therapies evaluation

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INTRODUCTION

Hematological malignancies are rare malignant disorders accounting for 6.2% of all deaths from cancers. For decades, chemotherapy was the only available approach for patients with advanced hematological malignancies but the effectiveness of such therapy has reached a plateau. This has prompted a search for targeted therapies with higher efficacy and lesser toxicities. Both antibodies and small-molecule compounds are therefore promising tools for target-protein-based cancer therapy. The emergence of targeted agents in clinic gives new efficient alternatives to patient in failure of reference treatments. Modeling human disease in rodents has become essential in biomedical research for the optimization of preexisting therapeutic modalities and the testing of novel ones.

In this study, we investigated the antitumor activity of MLN8054 (Aurora A kinase inhibitor), Glivec® (multi kinase inhibitor) and Mabthera® (monoclonal antibody against CD20) in such hematological malignancies disseminated in mice.

METHODOLOGY

❖ Test Substances

Adriblastine® (Pfizer, USA), Glivec® (Novartis, Switzerland), Mabthera® (Roche, Swizerland), Taxol® (BMS, USA), Velcade® (Janssen-Cilag, France) and Dexamethasone (Sigma, France) were purchased by ONCODESIGN. MLN8054 was supplied by Millennium Pharmaceuticals Inc.

❖ MTS Assays

The human cell lines were obtained from LGC promochem (France) and DSMZ (Germany). The resistant K-562-IMR cell line was selected from K-562 cell line by Oncodesign after coculture with 1µM Glivec®. K-562-IMR results in bcr-abl gene amplification and overexpression of MDR as resistance mechanisms.

The tumor cell lines were implanted during 24 hours in drug free-medium and then incubated for 96 hours with 10 concentrations of test substances. At the end of drug incubation, the effect of treatment on cell proliferation was evaluated by a MTS assay (1). The IC_{50} values (from at least three independent experiments) were calculated using the XLFit 3 software (IDBS, United Kingdom) from semi-log curves.

❖ *In vivo* experiments

Irradiated female SCID mice obtained from Charles River (France) were IV injected with hematological tumor cell lines at D0 (except for ARH-77 model). Isoflurane (Minerve, France) was used to anaesthetize the animals before IV cells injection, IV treatments and termination. The viability/behavior and body weights of mice were recorded every day and twice a week, respectively. All logistical issues of the study (dosing, collection, measurements…), raw data, lethality, behavior and results of autopsy were managed using Vivo Manager software (Biosystemes, Dijon). During the course of the experiment, animals were killed under anesthesia when they displayed significant signs of behavioral and/or physiological changes (signs of suffering, cachexia, weakening, difficulty moving or eating and/or 20 % body weight loss). ILS % (Increase Life Span) was expressed as following: ILS% = (T-C)/Cx100 (T: Median survival time of mice treated with drug C: Median survival time of control mice). Animal experiments were performed according to ethical guidelines of animal experimentation (2) and the English guidelines for welfare of animals in experimental neoplasia (3). All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon).

❖ Human cell detection in mice tissues

To study the human cells engraftment in mice, some mice tissues (bone marrow, ovary, brain, adrenal gland, kidney, liver, lung, spleen, spinal column, femur) were collected at various time points for subsequent FACS and histology analyses (HE staining). Cells suspensions were prepared from tissues either by mechanistic dissociation or by dispase/collagenase enzymatic digestion (Gibco, France). The human cells were detected by FACS analysis (CyFlow®, Partec, France) using an anti-human CD45 antibody (clone J.33, Ref A07783, Beckman Coulter, France). Tissues from healthy mice were used for background signal determination.

(1). BALTROP J.A. et al., Bioorg. Med. Chem. Lett., 1:611-614, 1991.
(2). 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.
(3). WORKMAN P. et al., UKCCCR guideline. Br. J. Cancer, 77: 1-10, 1998.

RESULTS - *In Vitro* Characterization

Sensitivity of human hematological tumor cell lines to chemotherapeutic agents (mean IC_{50} ± SD)

Cell line	Type	Taxol® IC_{50} (nM)	Adriblastine® IC_{50} (µM)	Glivec® IC_{50} (µM)	Velcade® IC_{50} (nM)
ARH-77	Plasma cell leukaemia	21.3 ± 28.6	0.1 ± 0.1	ND	6.6 ± 3.9
CCRF-CEM	ALL	28.7 ± 36.5	4.1 ± NA	48.6 ± 37.4	4.6 ± 1.5
CCRF-CEM/VLB*	ALL	> 100	> 100	85.9 ± NA	26.7 ± NA
Daudi	Burkitt's lymphoma	24.2 ± 21.9	0.4 ± 0.5	33.9 ± 17.8	4.4 ± 3.1
HL-60	AML	19.8 ± 57.1	3.0 ± 10.6	0.7 ± 0.4	16.6 ± 2.9
K-562	CML	7.6 ± 5.4	ND	0.7 ± 0.5	ND
K-562-IMR	CML	> 100	> 100	57.3 ± 35.2	ND
Namalwa	Burkitt's lymphoma	5.4 ± 1.2	30.6 ± 8.1	ND	4.0 ± 1.2
Raji	Burkitt's lymphoma	26.3 ± 11.9	ND	ND	6.4 ± 1.5
Ramos	Burkitt's lymphoma	ND	ND	ND	2.3 ± 0.5
RPMI 8226	Myeloma	5.2 ± 8.9	0.2 ± 0.3	47.8 ± 2.6	4.2 ± 2.2
RPMI 8226/Dox40	Myeloma	> 100	46.0 ± 19.6	> 100	9.4 ± 5.5

ND : Not done NA : Not applicable * Resistance factor to VLB of about 20 000-fold compared to CCRF-CEM

RESULTS - *In Vivo* Characterization

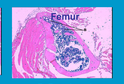
Survival time of SCID mice IV injected with human hematological tumor cell lines

Cell lines	Number of injected cells	Mean survival time ± SD (days)	Median survival time (days)
Ramos	$2x10^6$	22.0 ± 1.0	22.0
Namalwa	$1.25x10^6$	17.0 ± 0.0	17.0
K-562	$10x10^6$	44.1 ± 5.4	43.5
K-562-IMR	$10x10^6$	51.5 ± 11.3	47.0
Raji	$5x10^6$	21.0 ± 0.1	21.0
Daudi	$5x10^6$	40.2 ± 5.7	40.5
RPMI 8226	$10x10^6$	89.3 ± 4.1	91.0
RPMI 8226/DOX40	$10x10^6$	96.3 ± 13.9	99.0
ARH-77	$5x10^6$	38.2 ± 7.0	35.0
CCRF-CEM	$5x10^6$	88.3 ± 38.1	77.0
CCRF-CEM/VLB	$5x10^6$	103.3 ± 36.5	96.0
HL-60	$5x10^6$	70.6 ± 40.4	54.0

Histology of mouse tissues (x40)

Ramos

A: Meningis invasion
B: Bone invasion
C: Muscle invasion

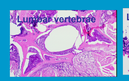


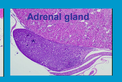
B: Bone invasion

D: Fat tissue invasion

A: Meningis invasion

Namalwa





FACS analysis of hCD45 positive cells in SCID mice tissues (%)

Model	Tissues \ Time (day)	D1	D8	D15	D20
Ramos	Bone marrow	0	2	19	40
	Ovary	1	0	0	7
	Brain	0	0	0	16
	Adrenal gland	0	0	0	16
	Kidney	0	1	0	0
	Liver	0	0	0	0
	Lung	0	0	0	0
	Spleen	1	0	1	0

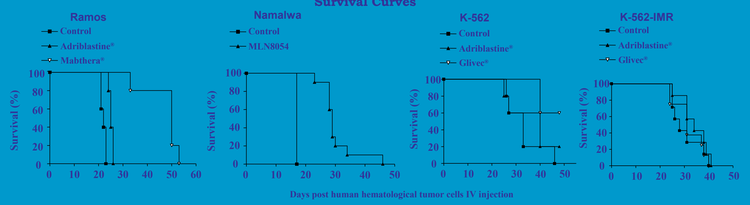
Model	Tissues \ Time (day)	D1	D11	D20	D35
K-562	Bone marrow	0	0	0	0
	Ovary	0	0	39	7
	Brain	3	0	2	11
	Adrenal gland	1	0	2	45
	Kidney	1	0	0	0
	Liver	0	0	0	0
	Lung	0	0	0	0
	Spleen	0	0	0	2

➢ In Ramos model, the human tumor cells were detected in bone marrow, brain, adrenal gland, spinal column and ovary,
➢ In K-562 model, the human tumor cells were detected in adrenal gland, ovary, brain and spleen,
➢ In Namalwa model, the human tumor cells were detected in adrenal gland, femur and spinal column

RESULTS - Effects of drugs on survival of mice (ILS%)

Cell line	Treatment	Dose (mg/kg)	Schedule	Route	Day of treatment initiation	ILS (%)
Ramos	Adriblastine®	2	Q4Dx3	IV	4	14
	Mabthera®	10	Q4Dx3	IV	4	127
Namalwa	MLN8054	30	BIDx21	PO	3	71
K-562	Adriblastine®	2	Q4Dx3	IV	4	0
	Glivec®	400	Q1Dx30	PO	4	>50
K-562-IMR	Adriblatine®	2	Q4Dx3	IV	4	0
	Glivec®	400	Q1Dx30	PO	4	0
ARH-77	Adriblastine®	2	Q4Dx3	IV	3	26
	Dexamethasone	1	(Q2Dx3)x2	IV	3	0

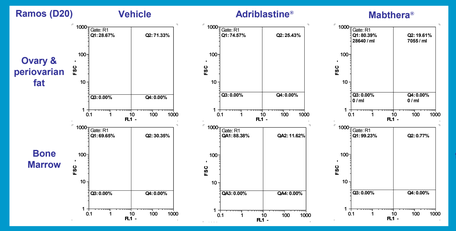
Survival Curves

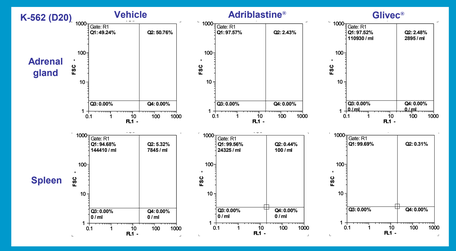


➢ The targeted therapies (Mabthera®, MLN8054 and Glivec®) showed a marked antitumor activity in the tested hematological human tumor models in mice.

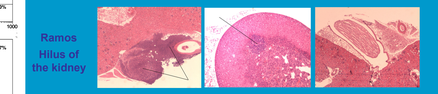
RESULTS - Engraftment of human cells in SCID mice

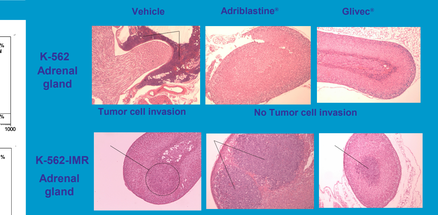
Engraftment of hCD45 positive cells in SCID mice tissues





Histology of mouse tissues





Cell line	Tissues	Bone marrow					Ovary					Adrenal gland					Brain				
	Time (day)	D11	D15	D20	D26	D50	D11	D15	D20	D26	D50	D11	D15	D20	D26	D50	D11	D15	D20	D26	D50
Ramos	Vehicle	3	5	24	FD	FD	5	5	43	FD	FD	0	3	48	FD	FD	0	1	3	FD	FD
	Adriblastine®	1	7	9	61	FD	2	28	27	50	FD	1	5	20	58	FD	0	1	1	6	FD
	Mabthera®	3	1	1	ND	3	4	1	15	ND	5	3	5	9	ND	38	0	1	1	ND	32

FD: Found dead

Cell line	Tissues	Kidney		Ovary		Adrenal gland		Brain		Spleen	
	Time (day)	D28	D40	D28	D40	D28	D40	D28	D40	D28	D40
K-562	Vehicle	2	FD	7	FD	42	FD	6	FD	3	FD
	Adriblastine®	1	9	2	8	2	7	2	3	0	1
	Glivec®	1	2	2	4	2	9	8	10	0	1

➢ The increase of survival times of Ramos and K-562 tumor bearing mice after Mabthera® and Glivec® treatment is correlated with a delay of tumor growth in specific tissues

CONCLUSIONS

✓ The targeted therapies (Mabthera®, MLN8054 and Glivec®) showed a marked antitumor activity in the tested hematological human tumor models in mice,

✓ These results showed that these models were relevant of the human disease and were reliable tools for preclinical evaluation of efficiency of new targeted therapies,

✓ The human cells engraftment analysis in these models was used as early marker for antitumor activity of new therapies (in accordance with ethical guidelines)