Biological characterization and non invasive PET imaging explorations of lymphatic dissemination in a human melanoma xenograft model

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Olivier DUCHAMP[1], Raphaël BOISGARD[2], Bertrand TAVITIAN[2], Philippe GENNE[1] and Nicolas GUILBAUD[1]

1) Oncodesign SA, Dijon, France; 2) In vivo imaging of gene expression laboratory, INSERM/CEA/SHFJ, Orsay, France

INTRODUCTION

The major cause of death from melanoma is related to metastases development, these secondary tumours being resistant to conventional therapies. Determining the metastatic spread of melanoma cells to the regional lymph nodes is essential in the assessment of prognosis and treatment options. Several human melanoma cell lines have been reported to metastasize in the immune-deficient mice, but lymph nodes metastatic rates were extremely low. To evaluate the potential of therapies against metastatic melanoma, we developed a novel human xenograft model in immunodeficient rodents (mice and rats) involving metastasis formation in multiple organs.

Positron emission tomography (PET) is a diagnostic imaging modality that allows the characterisation of disease based on altered metabolism. ^{18}F-fluorodeoxyglucose (FDG) is the most frequently used PET radiotracer. The sensitivity of the microPET® scanner led to the suggestion that FDG-PET might be used i) for initial non-invasive spreading assessment and, ii) for pharmacological treatment investigation in our experimental melanoma model.

OBJECTIVES

In vitro and _in vivo_ biological characterization of the CMEL-5 human melanoma cell line
To investigate the sensitivity of the CMEL-5 xenografts models to reference drugs
To evaluate the usefulness of PET imaging modality for the melanoma spreading and for the tumour response after BCNU treatment on nude rats

ORIGIN OF THE CMEL-5 MELANOMA CELL LINE

The LB1319-MEL cell line was purchased from Dr. B. Van Den Heynd (Ludwig Institute, Brussels) and was first established _in vitro_ from a metastatic malignant melanoma in a 72-year old caucasian male.
The CMEL-5 human melanoma cell line was originated from a brain metastasis induced by LB1319-MEL cells intravenously (IV) injected in nude mice. The CMEL-5 cell line was obtained by _in vivo_ selection for its high capacity to lymph nodes tropism when injected IV to nude mice.

METHODOLOGY

Immunocytometry analysis of the CMEL-5 cell line
CMEL-5 cells were grown as monolayer in complete culture medium (RPMI1640/10% FBS). Cells were trypsinized for cell suspension obtaining and 10^6 cells were incubated with a panel of antibodies to evaluate by Flow cytometry (FACScan) the expression of AlphaVBeta3 (LM609, Upstate, Charlottesville, USA) , VEGFR2 , IL-18 Receptor, VLA-4, CCR7 (R&D Systems, Mineapolis, USA), and CXCR4 (R&D Systems, Mineapolis, USA).

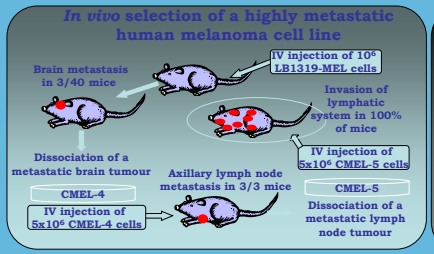
Experimental tumour models:
Female swiss-_nu/nu_ mice and NIH-_rnu/rnu_ rats 5-7 weeks old of age (Charles River, France) were used for these studies. To develop the disseminated tumour model 10^6 or 5.10^6 CMEL-5 cells were intradermaly (OT) injected in _Nude_ mice and _Nude_ rats respectively. To develop the orthotopic tumour model 5.10^6 or 10^7 CMEL-5 cells were IV injected in _Nude_ mice and _Nude_ rats respectively. The day of cancer cells injection was considered as the D0.

***In vivo* tumour growth inhibition studies**
The tumour sensitivity to chemotherapeutic agents such as carmustin (BCNU), temozolomide (TMZ), 5-Fluorouracil (5-FU) and taxol (TXL) was investigated. _Nude_ mice and _Nude_ rats bearing CMEL-5 orthotopic (OT) or disseminated (IV) tumours were randomized at D13-D21 according to tumour volume (for OT models) or to body weight (for IV models) to constitute groups of 5-6 animals. The treatment doses and schedules were indicated in the table below:

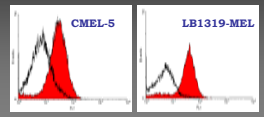
Animals	Tumour implant. site	Treatment	Treatment doses	Treatment adm.	Treatment schedule	Treatment start after cells graft
Nude mice	OT	BCNU TMZ 5-FU TXL	20 mg/kg/inj 50 mg/kg/inj 80 mg/kg/inj 15 mg/kg/inj	IV PO IV IV	Q7Dx3 Q3Dx5 Q7Dx3 Q7Dx3	D13 D19 D19 D19
Nude rats	OT	BCNU	20 mg/kg/inj	IV	Q7Dx3	D19
Nude rats	IV	BCNU	10 mg/kg/inj	IV	Q14Dx2	D21

Positron emission tomography
The PET imaging studies were performed on _Nude_ rats bearing IV induced CMEL-5 tumours on a microPET® FOCUS 220 scanner (Concorde Microsystems-CTI) which has a spatial resolution of 1.34 mm at the center of the field of view. Rats were injected with 37 MBq of ^{18}FDG via the tail vain. 45 min after injection, the rats were placed in the centre of the camera's field of view, and PET images were acquired during 15 min by bed position (3 positions for whole body acquisition). Images were reconstructed using the FORE-OSEM 2D algorithm and analyzed using the Asipro VM analysis tool (Concorde Microsystems- CTI). PET diagnostics were performed from D13 to D117 for 8 _Nude_ rats and from D40 to D97 for the 3 BCNU treated rats

In vivo selection of a highly metastatic human melanoma cell line



In vitro expression of $\alpha_v\beta_3$ integrins in CMEL-5 and parental LB1319-MEL cells



In vitro Analysis of CMEL-5 cells by cytometry

Parameter	Total Population of tested CMEL-5 metastatic cells
IL18 Rα	5.72 %
VEGFR-2	13.31%
VLA-4	37.51%
αVβ3	48.4%
α4β1	42.1%
CXCR4	4,6%
CCR7	97,97 %

IV-induced CMEL-5 tumour development in the *Nude* rats

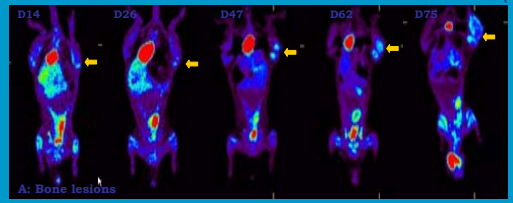
Table 1

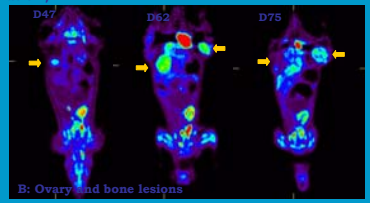
Time after Cells inj.	IP lymph nodes	Ovaries Adrenal gland	Brain	Bone	Lung
D20-D30:	0 / 0	0 / 0	0 / 0	25 / 12	25 / 0
D30-D50:	80 / 0	0 / 25	0 / 0	40 / 37	40 / 0
D50-D120:	100 / 12	80 / 25	60 / 0	60 / 37	20 / 0

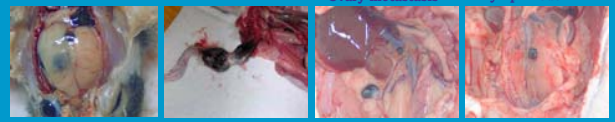
Table 1 represents the lesions incidence (%) diagnosed in several experiments by macroscopic observations (in white) and by FDG-PET images in one experiment (in red).

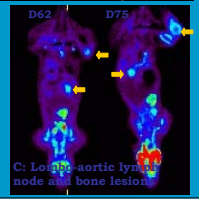
FDG injection in healthy Nude rats have shown a high FDG uptake in the thorax, abdomen, joint, and brain. This was a major obstacle for abdominal and thoracic lymph nodes small lesions diagnostic.

FDG-PET images was well adapted to diagnose bone and ovary metastases (images A & B) as earlier as macroscopic observations (Table 1)





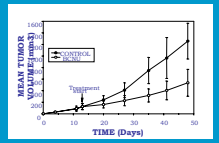




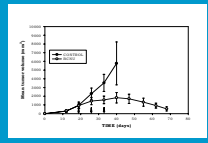
All PET images were confirmed by macroscopic examinations after humanized termination of the rats

In vivo Antitumour activity of a panel of anticancer drugs

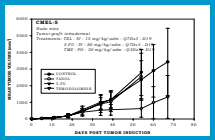
1: BCNU in OT CMEL-5 tumours in *Nude* mice



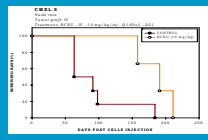
2: BCNU in OT CMEL-5 tumours in *Nude* rats



3: TXL, 5-FU, and TMZ in OT CMEL-5 tumours in *Nude* mice



4: BCNU in IV-induced CMEL-5 tumours in *Nude* Rats



BCNU inhibited the orthotopic CMEL-5 tumour growth in _Nude_ mice (T/C_{max} = 39%, _Fig.1_) and in _Nude_ rats (T/C_{max} = 44%, _Fig.2_). TMZ inhibited the orthotopic CMEL-5 tumour growth in nude mice (T/C_{max} = 18%, _Fig.3_). 5-FU and TXL didn't show any anti-tumour efficacy in orthotopic tumours in _Nude_ mice (Fig.3). BCNU increased the survival of IV-induced CMEL-5-bearing nude rats (ILS=148%, _Fig.4_)

FDG-PET study of BCNU efficacy in *Nude* rats

Groups	Nb of rats with positive PET lesions (D50-D75)
CONTROL	4 / 8
BCNU	0 / 3

Table 2: **Rats were IV injected with CMEL-5 cells at D0. Until D75, 50% of control rats were diagnosed with tumour lesions versus 0% of BCNU-treated rats.**

CONCLUSIONS

- **CMEL-5 is a novel and well characterized malignant human melanoma model**
- **CMEL-5 melanoma model in _Nude_ mice and rats recapitulates the metastatic spreading observed in the human pathology**
- **CMEL-5 cell line is a useful model to study _in vivo_ angiogenesis and interactions of tumour cells with their host microenvironment**
- **FDG-PET scanning is unable to detect small lymph node metastases in our CMEL-5 model. Only ovarian and bone lesions could be diagnosed with FDG-PET system. These results are in accordance with some clinical literature**
- **FDG-PET scanning could be used to investigate the efficacy of novel therapies in the CMEL-5 melanoma model**

BIBLIOGRAPHY
1) Belbochine T. et al., The Oncologist, 2002, 7:271-278
2) Mijahout GS. Et al., Journal of Clinical Pathology, 2002,
3) Swetter SM. Et al., Annals of Surgical Oncology, 2002, 9:646-653