Microbeam Radiation Therapy (MRT): how to find the best compromise between the sparing of normal tissues and the cure of a tumor ?

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AIM

-Reproducibility of the MRT experiment performed at BNL by Laissue et al [1]

-Optimisation of beam parameters and tumor implantation protocol

Matters ???

- Biological parameters → Reproducibility of cell culture protocol; Test of the influence of implantation modalities; Confirmation of tumor before irradiation
- Differences of the two sources → Difference of spectra… → Which impact on results?

INTRODUCTION

Principe of Microbeam Radiation Therapy

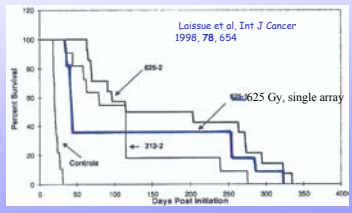
Alternation of 25 µm wide high doses zones (peaks-625 Gy) and 100 or 200 µm wide low doses zones (valleys-between 10 and 20 Gy) created by a microbeam collimator

Theoretically:

- **sparing** of normal brain tissue (possible regeneration of endothelium)
- Tumor **ablation** (no regeneration)

⇒ Effect enhanced by **differences in the development of the vascularisation** between tumor and normal tissues

Results of Laissue *et al.* in 1998 at the BNL



-Spacing between microbeams: 100 µm

-Median survival time of unirradiated controls: 20 days after tumor initiation

-Median survival time was extended in 625 Gy, single array irradiated rats by 24 days

-Death for unknown reasons after long term survival

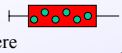
MATERIAL AND METHODS

IMPLANTATION

-10^3 9L cells in 5 µl

-10^4 9L cells in 1 µl

- injected into the right brain hemisphere



Male Fischer 344 rats

Tumor growth **14 days**

9 to 12 days

Future of the animals

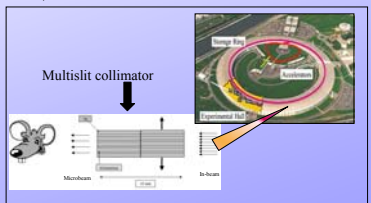
- Clinical and neurological signs are noted
- Rats are weighted 3 times / week
- After dead, rat brain is fixed in formalin
- Histology to discover dead causes (tumor regeneration or radiation damage of healthy tissues-pending)

Magnetic Resonance Imaging

- Sequence Turborare
- T_2 weighted
- Without contrast agent
- 3D mode

Diagnosis of tumor performed before irradiation with a 3 Tesla magnet

Irradiation of rat brains



Several protocols of irradiation:

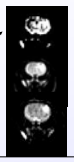
Series	Cells type	Protocol of implantation	Slit/Seam	Spacing	Direction of irradiation	Rats per series	Interval implantation-MRI
1	9L	10^4 c in 1 µl	Slit	200 µm	left to right	23	11 et 12 days
2	9L	10^4 c in 1 µl	Slit	200 µm	right to left	11	12 days
3	9L	10^4 c in 1 µl	Slit	100 µm	right to left	12	11 days
4	9L	10^4 c in 5 µl	Slit	200 µm	left to right	10	11 days
5	9L	10^4 c in 5 µl	Slit	200 µm	right to left	8	11 days
6	9L	10^4 c in 5 µl	Slit	100 µm	right to left	8	10 days

RESULTS/ DISCUSSION

1) 3 long term survivals:

- C03 (serie 1:10^4 cells 200µm L to R) still alive at 342 days
- C54 (serie 2:10^4 cells 200µm R to L) still alive at 230 days
- C95 (serie 3:10^4 cells 100µm R to L) dead 158 days a.i.

Presence of tumor checked by MRI



2) The rats with small size tumor (series 4, 5, 6) are not cured (this corresponds to the smallest number of cells injected i.e. 10^3 cells against 10^4 cells

3) The highest average in survival time (72.7 days) is the one cured with 100 µm spacing (10^4 cells)

4) Series cured with 200 µm spacing (10^4 cells) have not a very good average survival time but they both have a long time survival still alive (series 1 and 2)

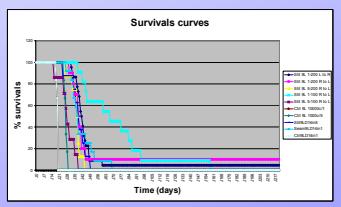
Séries	Median (days)	Average (days)	SEM	survivors	quantity of rats
1: Slit-9L-1-GaD-200	41,5	41,6	1,37	1	21+1
2: Slit-9L-1-DaG-200	38,5	37,9	1,48	1	9+1
3: Slit-9L-1-DaG-100	67	72,7	10,4	0	13
7: Seam-9L	37,5	41,1	3,32	0	12
L: Ctrl-9L-10^4-1	20	19,5	0,34	0	6
4: Slit-9L-5-GaD-200	38,5	38,25	2,64	0	8
5: Slit-9L-5-GaG-200	37,5	36,5	1,84	0	8
6: Slit-9L-5-DaG-100	28	28,4	2,7	0	7
H: Ctrl-9L-10^4-5	27	27	0,58	0	6

5) Rats irradiated at 100 µm (10^4 cells) had a lot of abnormal clinical signs (8 rats /11) and were not in good health with a constant weight.

6) Rats irradiated at 200 µm (10^4 cells) were in general good health and had not many abnormal clinical signs (4 rats on 32) . In addition, long term survivals took weight.

Hypothesis :

- MRT would be more efficient on tumor implanted with the highest cell density (=later stage tumor), due to an insufficient vascularisation in small sized tumors.
- Compromise to be found: (i) in case of 100 µm spacing, the tumor are cured but rats died/suffer from neurological disorders; (ii) in case of 200 µm spacing, rats died from their tumor, but survivors are in good clinical status.



Despite biology protocols very close the ones used by Laissue *et al.* (1), results are not optimum in term of survival (40% vs. 10%) although are close to recent ESRF results (Smilowitz *et al.* (2)), with 200 µm spacing. It may be due to spectra differences (BNL versus ESRF).

However

- Knowledge of the influence of implantation modalities on survival curves (frequently neglected in protocols)
- Crucial importance of the balance between irradiation with a 100 µm spacing (good resection of tumors) and a 200 µm spacing

PERSPECTIVES

• Focus on balance between 100 and 200 µm spacing in order to optimize survival curves by (i) at 200 µm spacing: increasing skin entrance dose or increasing microbeam size, (ii) at 100 µm : decreasing skin entrance dose

APPLICATIONS

• Combination to anti-angiogenic treatment in order to combine the action of MRT and chemotherapy
• Application to other tumor cell lines

Références :

[1] : Laissue *et al.*, Int. J. Cancer, 1998 [2] : Smilowitz et al., 94th annual meeting of the American Association for Cancer Research, 2003