Optimisation of combination of peptide receptor radionuclide therapy (PRRT) and temozolomide therapy using SPECT/CT and MRI in mice

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Purpose

For treatment of patients suffering from somatostatin receptor (SSTR) overexpressing neuroendocrine tumours, a combination of the successful peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues such as Lutetium-177 labelled octreotate ($^{177}$Lu-TATE), plus the alkylating agent temozolomide (TMZ) is most promising. Therapeutic responses however will be dependent on the applied therapeutic scheme. Including the order, dose and duration of therapies. We therefore aimed to study anti-tumour effects and other tumour characteristics in a preclinical setting using different treatment schedules using SSTR-expressing H69 xenograft-bearing mice. The level of SSTR expression and tumour perfusion was determined using small animal SPECT and MRI, respectively, both prior to and after therapy with PRRT and TMZ. Therapeutic responses and survival rates were included as the outcome parameters defining the optimal treatment protocol.

Methods and materials

Experimental setup

1 Imaging studies to study tumour characteristics during PRRT or TMZ treatment

Human SCLC derived H69 cells were inoculated in immune deficient NMRI nu/nu mice. Three groups of H69 tumour bearing mice were included in this imaging study. The PRRT group received a single dose of 50MBq $^{177}$Lu-TATE (100 MBq/2.75µg peptide) on d1 and the TMZ group was treated with 50mg/kg TMZ administered orally for 14d, starting at d1. The control group received saline.

Table 1: Overview experimental groups in imaging study

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mice</th>
<th>Treatment</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRT</td>
<td>4</td>
<td>50MBq $^{177}$Lu-TATE i.v.</td>
<td>7 x MRI, 1 x SPECT/ CT</td>
</tr>
<tr>
<td>TMZ</td>
<td>10</td>
<td>TMZ 50 mg/kg p.o. for 14d</td>
<td>7 x MRI, 7 x SPECT/ CT</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>Saline</td>
<td>4 x MRI</td>
</tr>
</tbody>
</table>

PRRT group(figure 1)
The $^{177}$Lu-TATE group (n=4) received a DCE-MRI baseline scan 2d prior PRRT, repeated at d4, 7, 10, 14, 20 and 28. A SPECT scan to determine uptake of $^{177}$Lu-TATE was performed 24h after administration.

**TMZ group** (figure 2)

The TMZ group (n=10) received one baseline MRI scan two days prior to TMZ treatment, followed by MRI once weekly for 4 weeks during and after TMZ treatment. Moreover, $^{111}$In-octreotide SPECT was performed to determine tumour SSTR2 expression, 1d prior to TMZ treatment and once weekly for 6 weeks during and after TMZ. Next to imaging, also $^{111}$In-octreotide in vitro autoradiography was performed to monitor SSTR expression in tumours from TMZ-treated mice, at different time points: at d-1, at d15, just after TMZ treatment and at d28, when tumours showed maximal therapy response, three mice where sacrificed to collect the H69 xenograft.

**Control group**

Non-treated mice (n=3) received 4 MRI scans; at d-1, 4, 7 and 11.

**2 Combination therapy of PRRT and TMZ**

Seven therapy groups of H69 tumour bearing mice were included to compare responses to different treatment schedules and compared with a placebo treated control group (Table 2).

**Table 2: Overview treatment experimental groups in combination therapy study**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TMZ</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>$^{177}$Lu-TATE</td>
<td>placebo</td>
<td>5</td>
</tr>
<tr>
<td>1: PRRT single</td>
<td>30MBq d1</td>
<td>placebo</td>
<td>8</td>
</tr>
<tr>
<td>2: PRRT double</td>
<td>30MBq d1, 30MBq d15</td>
<td>placebo</td>
<td>8</td>
</tr>
<tr>
<td>3: TMZ</td>
<td>-</td>
<td>50mg/kg for 14d from d1</td>
<td>9</td>
</tr>
<tr>
<td>4: PRRT + TMZ at d15</td>
<td>30MBq d1</td>
<td>50mg/kg for 14d from d15</td>
<td>8</td>
</tr>
</tbody>
</table>
5: PRRT + TMZ  30MBq d1  50mg/kg for 14d  8
from d1

6: TMZ + PRRT at  30MBq d15  50mg/kg for 14d  10
from d1

Images for this section:

Fig. 1

Fig. 2
Results

1. Imaging studies

Effect of single agent treatment on tumour growth

Treatment with either $^{177}$Lu-TATE or TMZ resulted in transient reduction in tumour size/volume. However, the kinetics of these effects were different for the two protocols (Figure 3).

Tumour perfusion during PRRT or TMZ treatment

Control tumours showed low perfusion values (k-trans, Fig 4), decreasing slowly throughout tumour growth/proliferation. Tumours subjected to TMZ and PRRT initially showed a fast decrease in the median perfusion parameter k-trans, reaching minimum values on day 4 (PRRT) or 5 (TMZ).

SSTR expression prior to and after TMZ treatment

In vitro autoradiography results using frozen sections showed no difference in SSTR expression of H69 xenografts, when collected before (d0) or after (d15 and d28) TMZ treatment (Figure 5).

Uptake of $^{111}$In-octreotide during and after TMZ treatment

Based on SPECT images, the amount of $^{111}$In-octreotide tumour uptake was quantified which showed an increase until d14 (last day of TMZ treatment) and a decline afterwards (Figure 6). Since the SSTR-expression was not increased (Figure 5) improved tumour perfusion may have led to optimal uptake of radiolabelled somatostatin analogues.

2. Therapy study

Responses to the different treatment schedules

All mice from the control group, but also from the groups treated with either single $^{177}$Lu-TATE (group 1 and 2) or TMZ (group 3), eventually had to be euthanized due to excessive tumour growth (Figure 7). In the group receiving TMZ treatment 14 days after administration of $^{177}$Lu-TATE (group 4) only 57% of tumours reached a volume
of 1800mm$^3$ before the end of the study at d123. This was even only 25% in group 5 when $^{177}$Lu-TATE and TMZ were combined from the start. The best result was obtained however in group 6 when $^{177}$Lu-TATE was administered after 14d TMZ treatment, because only 10% of tumours reached a volume of 1800mm$^3$.

_Tumour uptake of $^{177}$Lu-TATE_

In the therapy study the average tumour uptake of $^{177}$Lu was 1.73 kBq/mm$^3$ 24h after administration of $^{177}$Lu-TATE, without any previous treatment. When a second dose of $^{177}$Lu-TATE was given after 14d, a comparable tumour concentration of 1.68 kBq/mm$^3$ was obtained. However, in mice receiving $^{177}$Lu-TATE on d15 after initial TMZ for 14d a significantly higher uptake of 2.24 kBq/mm$^3$ was found (p=0.015) (Figure 8).

Images for this section:

**Fig. 3:** Effect on tumour size of single agent treatment. The control group (black line) received saline, the PRRT group was treated on d1 with 50MBq $^{177}$Lu-TATE (blue line) and the TMZ group (red line) was treated for 14d with orally administered TMZ at a dose of 50mg/kg.
Fig. 4: Tumour perfusion in PRRT and TMZ treated mice.
**Fig. 5:** SSTR expression of H69 tumours treated with TMZ. Quantification of SSTR density in frozen sections of H69 tumours using in vitro autoradiography. The amount of radioactivity is expressed in density light units (DLU)/mm². SSTR expression was determined at d 0, d15 (one day after TMZ treatment) and d28 when tumours reached a minimal volume after TMZ treatment.

![Graph showing SSTR expression over time](image)

**Fig. 6:** Tumour uptake of 111In-octreotide in mice treated with TMZ. At each time point the average tumour concentration of 111In-octreotide was determined 24h after administration, based on SPECT images. Mice were treated with TMZ from d1 until d14. Tumour uptake of 111In-Octreotide increased during TMZ therapy and peaked at day 14. After TMZ treatment had ended the uptake of 111In-Octreotide started to decrease.
Fig. 7: Percentage of tumours <1800mm³. The control group was treated with placebo, 30MBq 177Lu-TATE was i.v. administered at d1 for group 1, 4 and 5, at d15 for group 6 and both at d1 and d15 for group 2. TMZ was administered orally once daily for 14d at a dose of 50mg/kg from d1 for group 3, 4 and 6, but starting at d14 for group 5.
Fig. 8: Average tumour concentration of $^{177}$Lu-TATE 24h after administration, based on SPECT images. Red bar: average tumour concentration of $^{177}$Lu-TATE in all mice receiving $^{177}$Lu-TATE at day 1 (without any pre-treatment). Green bar: average tumour concentration of $^{177}$Lu-TATE in mice receiving $^{177}$Lu-TATE after a treated with TMZ for 14d. Blue bar: average tumour concentration of $^{177}$Lu-TATE after a second dose of $^{177}$Lu-TATE, administered 14d after the first dose.
Conclusion

Pre-treatment with TMZ improved the therapeutic effects induced by PRRT using $^{177}$Lu-TATE in H69-bearing mice, consistent with the maximum values of the perfusion parameters of the tumour leading to increased uptake/retention of $^{177}$Lu-TATE as well as better oxygenation facilitating the anti-tumour effects. Therefore, TMZ treatment prior to PRRT might be the best clinical practice to increase tumour response in NET patients.

Molecular imaging techniques applied in this study to determine tumour perfusion and uptake of radionuclides were very important to optimize the strategy for tumour treatment using the combination of two therapeutics. This is an excellent example on how the application of multimodality imaging techniques contributes to the development of optimized personalized medicine.

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