Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eTable 1. Breakdown of gastrointestinal events shown in Table 2

<table>
<thead>
<tr>
<th>Event</th>
<th>BSC alone</th>
<th>ADI-PEG20 &amp; BSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1-2</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Anorexia/weight loss</td>
<td>4 (17)</td>
<td>10 (23)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3 (12)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (4)</td>
<td>7 (16)</td>
</tr>
<tr>
<td>Constipation</td>
<td>2 (8)</td>
<td>8 (18)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Indigestion/dysphagia</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

1. Grade 4
eFigure 1. ADI-PEG20 administration. Number of cycles reached by each patient (upper), and total number of injections given to each patient (lower). Some patients reached a certain cycle but may not have had all 4 injections within it.
Males

<table>
<thead>
<tr>
<th>Time since randomisation (months)</th>
<th>% alive and progression-free</th>
<th>Hazard ratio 0.60, 95% CI 0.33-1.10</th>
</tr>
</thead>
</table>

No at risk:
- BSC: 19
- ADI-PEG20: 36

Females

<table>
<thead>
<tr>
<th>Time since randomisation (months)</th>
<th>% alive and progression-free</th>
<th>Hazard ratio 0.38, 95% CI 0.11-1.36</th>
</tr>
</thead>
</table>

No at risk:
- BSC: 5
- ADI-PEG20: 8

(interaction p-value was 0.50 for PFS and 0.52 for OS).

eFigure 2. Treatment effect of ADIPEG-20+BSC versus BSC alone according to sex (among n=55 males, and n=13 females).
(interaction p-value was 0.95 for PFS and 0.56 for OS).

**eFigure 3. Treatment effect of ADIPEG-20+BSC versus BSC alone according to whether patients had prior chemotherapy (n=40) or not (n=28)**
**eFigure 4.** Statistically significant (p=0.025) hypomethylation in the gene body (intron 1) of the ASS1 gene was identified in ASS1 negative (n=38) compared to ASS1 positive (n=17) mesotheliomas by immunohistochemistry (IHC). In contrast, methylation at the ASS1 transcription start site (TSS) or promoter was not observed in the clinical samples, while this was seen in all ASS1-negative mesothelioma cell lines tested (data not shown), except in the E58 cell line which mirrored the patient samples (shown in grey). Analysis of methylation was performed at 15 individual CpG loci across the ASS1 gene for negative vs positive cases using the Illumina Infinium 450K methylation array.

P-values were calculated using regularized Student t-tests with correction for multiple testing, given the 15 CpGs examined. We sought significant differences in DNA methylation for each CpG assayed in ASS1 using the regularized t-tests, where sample variances were shrunk by computing empirical Bayes posterior means using the *limma* package [Smyth GK, Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, 397-420 (2004)] in *R*; resultant p-values were corrected to account for the 15 CpGs examined by applying a false discovery rate (FDR) of 0.05.
eFigure 5. Comparison of overall survival between \( n=25 \) ASS1 negative patients (registered but not randomized); \( n=81 \) ASS1 positive patients (registered); and \( n=24 \) ASS1 negative patients (randomized to BSC alone in the clinical trial; in the main trial analysis 2 were censored just after randomization when they withdrew, but not censored in the figure above). The respective number of deaths were 22, 60 and 24. The corresponding restricted mean survival times were 8.8, 17.0 and 12.7 months. The curves are statistically significantly different (logrank \( p=0.001 \)).
eFigure 6. Patient self-assessment of QoL using the Lung Cancer Symptom Scale
eFigure 6 (cont.)

Pain

Lung symptoms

Ability to do normal activities

Rate QoL today

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Each sub-domain has a score range of 0-100, where 100 is the worst rating. The range for the total symptom score is 0-600, and 0-800 for the total score. Positive differences indicate that QoL got worse from baseline to 2 or 3 months post-randomization. Several patients had missing values for individual items on the questionnaire, and the total scores were only calculated if all items were completed. Although QoL assessments were scheduled for the end of month 2 and 4, most patients completed the second assessment closer to 3 months post-randomization.
eFigure 7. Observer assessment of QoL using the Lung Cancer Symptom Scale

Each sub-domain has a score range of 0-100, where 0 is the worst rating. The range for the total score is 0-500. Negative differences indicate that QoL got worse from baseline to 2 or 3 months post-randomization. Although QoL assessments were scheduled for the end of month 2 and 4, most patients completed the second assessment closer to 3 months post-randomization.
Figure 8. Plasma arginine, citrulline and anti-ADI-PEG20 antibody titer. Blood samples in the ADI-PEG20 + BSC arm were taken on the day of treatment and just prior to drug administration.
eFigure 9. PFS and duration of arginine deprivation in ADI-PEG20 patients (upper & middle figures), and PFS and baseline arginine in BSC alone patients (lower figure). In the upper figure, the correlation becomes 0.52 (p=0.006 after excluding the possible outlier with PFS>80 weeks. [PFS can be considered as a continuous measure because all patients had progressed]. Out of the 44 ADI-PEG20 patients, 1 had no arginine values and for another it was not possible to ascertain arginine deprivation (hence these 2 were excluded). Out of the 24 BSC alone patients, 3 were excluded because they had no samples or no baseline arginine.
eFigure 10. ASS1 expression at baseline and upon disease progression at 8 months, following ADI-PEG20 treatment in a patient with mesothelioma.

Note there is persisting downregulation of ASS1 in both tumor and endothelial cells in the progression biopsy indicating alternative mechanisms of acquired resistance to ADI-PEG20. Endothelial ASS1 (baseline) is a positive internal control. Additional controls not shown.