Treating Anaplastic Thyroid Carcinoma with an Oncolytic Adenovirus
Honey V. Reddi¹, Aliana J. Reichert-Eberhardt¹, Evanthia Galanis², Bryan McIver¹, Stefan K.G. Grebe³, John A. Copland², and Norman L. Eberhardt¹. ¹Dept of Medicine/Division of Endocrinology; ²Dept of Oncology, and ³Dept of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; ²Dept of Cancer Biology, Mayo Clinic, Jacksonville, FL

Background
Anaplastic Thyroid Carcinoma (ATC) is the most lethal form of thyroid cancer (life expectancy 6 months after diagnosis). Currently, no satisfactory form of treatment exists.

It may be possible to treat ATC with the use of adenoviruses and agonists of the PPARgamma cell pathway

Onyx-015 is a modified adenovirus that kills cells with a deficient p53 pathway. However it can also kill healthy cells

Onyx-411 kills cells that are deficient in the pRb (retinoblastoma) pathway. It is less toxic, and kills fewer healthy cells

PPARgamma is a cell signaling pathway. Agonists of this pathway have been shown to induce apoptosis in ATC cells

Rosiglitazone is a PPARgamma agonist. Use of Rosiglitazone aids the PPARgamma signaling pathway in inducing apoptosis

Hypothesis: Synergistic cancerous cell killing will occur with the combination of either Onyx-015 or Onyx-411 and Rosiglitazone

Materials and Methods
The effectiveness of Onyx and Rosiglitazone were determined by measuring cell viability.

Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT reacts with a mitochondrial dehydrogenase enzyme, resulting in a purple formazan product. The amount of formazan can be read using a spectrophotometer.

Formazan=mitochondrial activity=alive cells.

Non-cancerous Primary Thyroid cells were the negative control to determine how the treatment would affect normal tissue

ATC cell lines used were ARO, DRO, BHT101

N-Thyori immortalized cell line was tested to see if Onyx or Rosiglitazone had any effect upon it

MCF-7 Breast cancer cell line has been previously proven to be killed by Onyx and is the positive control

Cells were plated in 24 well plates with 2x10⁴ cells/well

Virus moi=50

Rosiglitazone added at 100nm/well

Ran MTT assay after 24 and 72 hours after plating cells

Results

Percent Viability of Cell lines at 24 and 72 hours after plating

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>24hrs</th>
<th>72hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMARY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHT101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percent Viability compared to the uninfected cells

Conclusions
Primary thyroid cell lines were not adversely affected by Onyx-015, Onyx-411, or Rosiglitazone. This shows that the virus and Rosiglitazone for the most part do not harm normal cells

Cancerous cell lines yielded varying results upon use of Onyx-015, Onyx-411, or Rosiglitazone

N-Thyori cell lines were killed by all but Onyx-015

The MCF-7 cell line shows low viability at 24 hours but then mostly rebounded by 72 hours. It is believed that the virus killed cells around 24 hours but then lost effectiveness by 72 hours

Rosiglitazone in combination with Onyx-411 had consistent, increased efficiency in cancerous cell killing

Continuations
The experiment will proceed to the use of transgenic mice for an in vivo look at the effects of Onyx and Rosiglitazone on ATC

Support
This experiment was funded by the Mayo Clinic, Rochester, MN