In just these 20 genes the combinations of mutations, copy number changes are vast.

In order to make use of these complex data one needs to create a statistical model based on a clinical training data set.

Genotypic signatures cannot be trained to recognise drugs that are never used in the clinic to treat a particular cancer.

The degrees of freedom in the statistical model make it very prone to over fitting the training set.
We only have one parameter in our data set that needs calibrating against clinical response, using a clinical training set, and that is *in vitro* drug dose.
A 3D Cell Death Assay

Originally developed using InSphero’s GravityTRAP™ specifically designed for scaffold-free microtissue culture.

- 3D Liver model for toxicity
- 3D Tumour microtissues for anti-cancer drug testing

HepG2 microtissues exposed to 10uM compounds for 7 Days

- HCA allows measurement of both tissue shrinkage and cell death within the tissue.
- Analysis in 96 well plates increases throughput allowing dose responses to be performed or the number of compounds tested increased.

- Blue- Nuclei staining
- Red- permeability stain
• Response is highly patient-dependent with some patients sensitive to the majority of treatments others highly insensitive to all treatment.
• Background cell death is a critical factor when looking at these particular heatmaps of multiple patients together.
• Background cell death is low in FBS, but in the selective media it can vary suggesting a subpopulation of ‘normal’ cells are being eliminated.
• Sensitive to Doxorubicin

• Gemcitabine

• Topotecan (in FBS only)

• First example of how we could improve our growth media to make the assay more predictive.
### Patient response matchup with assay prediction

**Green - Correct prediction**

**Pink - Incorrect**

**Blue highlights represent actual clinical responders**

<table>
<thead>
<tr>
<th>Responder? Identifier</th>
<th>Type</th>
<th>When Sample was taken</th>
<th>Response?</th>
<th>Prediction correct?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1901</td>
<td>Stage IIIa high grade endometrioid adenocarcinoma of ovarian type</td>
<td>post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>1967</td>
<td>stage 3c high grade serous</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>2170</td>
<td>poorly differentiated carcinoma of ovarian origin with omental mets</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>2175</td>
<td>Stage 4, mixed endometrioid and high grade serous</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>2218</td>
<td>Colon, Metastatic sigmoid cancer</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>196T1 (B27)</td>
<td>Ovarian</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>2118</td>
<td>Stage 3c/4 high grade serous adenocarcinoma of ovarian or primary peritoneal origin</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C001952</td>
<td>Stage 3c primary peritoneal ovarian</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C001952T2AFa</td>
<td>Psammoma carcinoma of ovarian</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002075 TAFA</td>
<td>Stage 3c/4 adenocarcinoma of primary ovarian</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C001595 TAFA</td>
<td>Unknown origin</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002220 TAFA</td>
<td>Stage 4 ovarian/peritoneal cancer</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002225TA2FA</td>
<td>Stage 3c High grade serious ovarian fallopian tubes/primary peritoneal carcinoma</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002227</td>
<td>Stage 3/4 Low grade serous carcinoma</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002284T1</td>
<td>Stage 3c ovarian high grade serous carcinoma</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002287TAFA</td>
<td>High grade serous ovarian adenocarcinoma</td>
<td>Pre treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002346TAFA</td>
<td>Primary tumour stage 4 ovarian cancer, Post treatment</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002375TAFA</td>
<td>Stage 3c low grade serous ovarian carcinoma</td>
<td>Mid treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002391TAFA</td>
<td>Relapsed stage 3C high</td>
<td>Mid treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>C002406T1</td>
<td>Recurrent high serous carcinoma</td>
<td>Post treatment</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>2112</td>
<td>High grade serous adenocarcinoma</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002747TAFA</td>
<td>Stage IIIc ovarian cancer</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002928</td>
<td>Carcinoma of the ovary, at least stage 3c primary/peritoneal Cancer/Ovarian stage 3c, poorly differentiated high grade adenocarcinoma</td>
<td>Mid treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002202TAFA</td>
<td>Stage 3C high grade serous carcinoma</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002347</td>
<td>poorly-differentiated carcinoma</td>
<td>Pre treatment</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>C002350TAFA</td>
<td>Poorly-differentiated carcinoma of the ovary</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002365TAFA (naive)</td>
<td>Stage 3C high grade serous carcinoma</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>C002401TAFA</td>
<td>High grade serous adenocarcinoma FIGO stage 3c</td>
<td>Pre treatment</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Cancer of unknown primary**

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**Imagen Biotech**

See more than images
Cancer very resistant to standard chemotherapies
This patient was analysed early in our development when we were comparing different culture media.

Red line response is our own proprietary media. Cancer appears to respond to Adriamycin but only at high doses.

Cancer may also be sensitive to docetaxel, gemcitabine and paclitaxel.
Patient 2138 Received

(Epirubicin, Oxaliplatin & Capecitabine) 16/06/2014 - 03/10/2014

Patient passed away 22/10/2014
Summary of Assay Predictive Rate

- 28 samples – 27 correct predictions (overall prediction 96%)
- All predictions indicating positive response were correct (7/7 – 100% specificity)
- Only 1 prediction indicating no response was incorrect
- The rest were correct (7/8 – 88% sensitivity)

Why do we out perform earlier *in vitro* cell death assay studies?

**Growth medium:** Our data demonstrates cells grown in FBS have a lower prediction rate of *in vivo* tumour response.

**Timing:** The assay needs to be performed immediately.
Sample C002287: Our assay showed correctly there would be no response. Stable cell line derived after several months in FBS showed incorrectly it would respond. This satisfies the strongly held position that cells change when you grow them on plastic long term.

**Assay:** We measure cell death directly not a surrogate marker of cell count.

Overall the 1990s data did show prediction of response but not improvement in survival (PM is only as good as the best drug you have available to treat the patient)
If you used our assay with standard licensed therapies then you will not have the statistical power to see significant shift on overall survival curves and this is the mistake that was made in the 1990s.
Responder versus non responder

Best licensed chemotherapy for patient G

Best response in larger 56 chemotherapy screen for patient Y
The Drugs Don’t Work they just make you worse … (The Verve, 1997)
The Drugs Don’t Work they just make you worse … (The Verve, 1997)
Larger Screen with 54 chemotherapies

- 2D measure is % cell death.
- Each block represents the mean response across a log scale dose response curve.
- **Heat map code:**
  1. Green = all data where death is below the 50 percentile.
  2. Red = all data where death is above the 97 percentile.
  3. 51-97 percentile coded in green/red colour mixtures proportional to the percentile value.
Could this be an assay false positive?
• Can use the data set a group of patients to address the question of *in vitro* assay false positives. If heterogeneous responses are observed for a particular drug in different patients then this suggests the assay is picking up true patient differences in chemosensitivity profiles for the particular drug under study (as shown below).

• Disturbingly these data predict that Bortezomib will work in only a subset of ovarian patients so how this will translate in a clinical trial is uncertain.
Phase I Clinical Trial – Bortezomib with Carboplatin

- 47% response rate
- 2 out of 15 complete responders
- 1 in platinum sensitive 1 in platinum resistant group

Conclusion of study is very positive and recommends progression to phase 2 trial

“The preclinical activity of bortezomib makes it a promising agent for combination therapy in overcoming chemotherapy resistance. The CR seen in this study in a platinum-resistant patient is also encouraging.”
Overall conclusion response rate no different from doxorubicin on its own so did not recommend further use.

“Our data suggests that bortezomib does not work as well when combined with doxorubicin.

“Conclusions: The combination of bortezomib and PLD was well tolerated, but the antitumor activity is insufficient to warrant further investigation in ovarian cancer.”
In Vitro results of Bortezomib versus Bortezomib & Doxirubicin

- Large error bars in 2D and 3D bortezomib because of the heterogeneous response of different patients to bortezomib (see slide 14 for example)

- In all 3D samples bortezomib and doxorubicin never worked better than bortezomib on its own.

- Both 2D and 3D data suggests that it is naïve to assume that drug combinations will never interfere with each other.
**Dasatanib** tested in an 8 Ovarian cell line panel

- 6 non-responders, 1 medium responder and 1 super responder.
- Would this pattern repeat in ovarian patients?
Ovarian cell lines treated with Dasatinib* (a BCR/Abl and Src inhibitor)

2 responders from 8 cell lines

We therefore hypothesized that we would get some responders in our Ovarian Panel from patients

There are clearly a subpopulation of patients that could benefit greatly from this drug, however they never receive them or if they do it is simply a calculated guess

* Bristol-Myers Squibb and sold under the trade name Sprycel. Dasatinib is an oral multi-BCR/Abl and Src family tyrosine kinase inhibitor approved for first line use in patients with chronic myelogenous leukemia (CML)[1] and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL).
Initial PDX Match Work

• Have received two frozen samples from a large company that specialised in PDX models.

• The first did not work, from the second we produced a good data set of 56 different chemotherapies.

• The second sample had been frozen for 8 years.

• Our match up with the PDX response was good.

• We will continue to work with this company hopefully moving towards a prospective treatment study where our data is used to directly influence the chemotherapy treatment of the mice.
Initial PDX Match Work

**In Vitro Data**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Schedule</th>
<th>% TGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel</td>
<td>20 mg/kg</td>
<td>iv</td>
<td>q4dx3</td>
<td>33</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1.5 mg/kg</td>
<td>ip</td>
<td>qdx5</td>
<td>20</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>20 mg/kg</td>
<td>iv</td>
<td>q7dx3</td>
<td>78</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>5 mg/kg</td>
<td>iv</td>
<td>q7dx3</td>
<td>59</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>25 mg/kg</td>
<td>iv</td>
<td>q7dx3</td>
<td>44</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>30 mg/kg</td>
<td>ip</td>
<td>q7dx3</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1.5 mg/kg</td>
<td>ip</td>
<td>qdx5</td>
<td>79</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>40 mg/kg</td>
<td>ip</td>
<td>q3dx4</td>
<td></td>
</tr>
<tr>
<td>Carboplatin</td>
<td>30 mg/kg</td>
<td>ip</td>
<td>q7dx3</td>
<td></td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>200 mg/kg</td>
<td>ip</td>
<td>qdx5x2</td>
<td>0</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>50 mg/kg</td>
<td>po</td>
<td>qdx28</td>
<td>66</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>10 mg/kg</td>
<td>iv</td>
<td>q4dx6</td>
<td>13</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>7.5 mg/kg</td>
<td>ip</td>
<td>q7dx4</td>
<td>58</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>35 mg/kg</td>
<td>po</td>
<td>qdx23</td>
<td>61</td>
</tr>
</tbody>
</table>

**Drug Comparison C**

- **In ovarian responders only see response @ top cisplatin dose**

**Complete Matchup**

- Erlotinib HCl (OSI-744)
- Gefitinib (Iressa)
- Lapatinib
- Paclitaxel + ve assay responder

**Reasonable Matchup**

**Possible Mismatch**
Next Steps

• We want to publish the data with our clinical colleagues as co-authors as soon as possible. Part this process will involve working closely with them to audit our matchup data and try to, as best we can mimic Recist categories even though these patients were not part of a formal clinical trial.

• We now want to expand quickly into other cancers especially finalising an upstream workflow for the collection of solid tissue from theatre.

• Work with a few surgeons in the MRI to tweak upstream workflow of tissue collection from theatre to Imagen Biotech.

• The next cancers we want to look at in order of preference are:
  1. Solid ovarian cancer (so we can compare data with ascites source).
  2. Malignant melanoma (because of the BRAF hyper-responder mutation).
  3. Cancer of Unknown Primary (CUP) because it does not have a clear chemotherapy protocol for treatment.
  4. Breast Cancer

• A prospective PDX animal study to validate our assay’s off-license predictive power.
Acknowledgements

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Dr Sacha Howell
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Christie Biobank
ThermoScientific

University of Manchester

Deniz Beyit & Mat Milner

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