Therapies for Overgrowth Disorders of PI3K-AKT Signalling Pathway

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Disclaimer

• A number of photographs included in Victoria’s original presentation have had to be removed, as they are unpublished patient images.
An Undiagnosed Overgrowth Condition
Fatty Overgrowth of Legs and Abdomen
Probable Mosaic Overgrowth Disorder
Genetic Investigation: Whole Exome Sequencing: Arm vs Leg

Biopsy 1 – “normal” arm tissue

Biopsy 2 – “overgrown” leg tissue
Mosaic Variant in *PIK3CA* Identified Exclusively in Legs

```
His/Leu 1047
CATTCATG

LEG

ARM
CACAATCATG 1047
His
```

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Mutation Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg Fibroblasts</td>
<td>50%</td>
</tr>
<tr>
<td>Arm Fibroblasts</td>
<td>0%</td>
</tr>
<tr>
<td>Bone</td>
<td>8%</td>
</tr>
<tr>
<td>Fat</td>
<td>39%</td>
</tr>
<tr>
<td>Fibrous Tissue</td>
<td>32%</td>
</tr>
<tr>
<td>Muscle</td>
<td>33%</td>
</tr>
<tr>
<td>Skin</td>
<td>24%</td>
</tr>
<tr>
<td>Blood</td>
<td>0%</td>
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*p.PIK3CA.His1047Leu*
PIK3CA encodes p110α subunit of phosphoinositide 3-kinase (PI3K)
Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA

De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly

De novo AKT3, PIK3R2 and related megalencephaly

De novo CCND2 mutations leading to stabilization of cyclin D2 cause megalencephaly polymicrogyria-polydactyly-hydrocephalus syndrome

LOVES Syndrome

De novo mutations in PIK3CA

Burns, Kym M, Bonnie A. Israel, and Ezaki, 2, 6
PIK3CA-related Overgrowth Spectrum
Segmental Overgrowth Study

- REC approval 2013
- Current recruitment:
  - 270 subjects
  - 21 different countries
  - 50% children < 17yrs
- 90 new genetic diagnoses (31%)
- Musketeer’s
- NHS England commissioning
- On Facebook/Twitter!

www.overgrowthstudy.medschl.cam.ac.uk
Potential Therapies

**PI3K/ p110α inhibitors**
- Phase II/III – Cancer trials

**AKT inhibitors**
- Phase II/III – Cancer trials

**Dual inhibitors e.g. PI3K/AKT: PI3K/mTOR: mTORC1/2 kinase**
- Phase II/III – Cancer trials

**mTORC inhibitors (rapalogs)**
- Available since 1990s
- Long-term use; established safety profile
Pre-Clinical Studies with Sirolimus in PIK3CA Mutant Dermal Fibroblasts

**Sirolimus 4ng/ml 96hrs**

<table>
<thead>
<tr>
<th>PIK3CA</th>
<th>WT</th>
<th>H1047L</th>
<th>H1047R</th>
<th>G1049R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cntrl</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N99</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Calnexin**

- 70kDa
- 60kDa
- 90kDa

**pp70S6**

- pp70S6
  - Thr389

**pAKT**

- pAKT
  - Ser473

**Cell Proliferation Assay**

- BrdU incorporation
- 5% FCS
- Sirolimus 4ng/ml added

**Relative BrdU incorporation**

- Controls n=3
- PIK3CA mutants n=3
- PIK3CA mutants + sirolimus 4ng/ml

**ELISA: pp70S6K**

- Sirolimus treated 96 hours

- Control
- PIK3CA mutant cells, n=3

**IC50 = 0.7 ng/ml**

**Relative phosphorylation p70S6K**

- [Sirolimus] ng/ml 96 hours

**ELISA pAKTser473**

- Sirolimus treated 96 hours

- Control
- PIK3CA mutant cells, n=4

**IC50 = 0.7 ng/ml**
Genetically Engineered Mouse Model of Mesodermal Pik3ca$^{H1047R}$ Mosaicism

In collaboration with S Castillo, B Vanhaesnbroeck UCL Cancer Institute, London

Off-Licence Therapy in an Affected Proband

*DXA estimated
Response to Sirolimus

Mean sirolimus plasma concentration = 3.5 ng/ml
Vd = 2.2 L/Kg
Sept 2012: Pre treatment   6 months sirolimus treatment   12 months sirolimus treatment

p.PIK3CA.His1047Leu identified in biopsy specimen
-17% mutation burden
Leptin as a Biomarker?
Clinical Trial with Sirolimus

- Purpose: Evaluate efficacy of sirolimus therapy in PIK3CA-related overgrowth
- Open label, uncontrolled, non-randomised, pilot trial
- 3 independent, identical, parallel trials UK, US, France

![Diagram showing study timeline with key events and time points.](image)
Trial Challenges: Rare Disease

- Limited overview of natural history
  - Pilot trial rather than RCT
- 10 patients in the UK
  - Collaboration with USA/France
- Participants scattered throughout UK
  - Blood tests for safety local to participants
  - NHS trusts no capacity!
  - GPs – CCG approval required – England only!
- Creep in off-licence prescription

- Collaborate
- Minimise study interventions/blood tests
- Private sector involvement
- Standardised agreement for blood tests in rare disease trials in UK?
Trial Challenges: Funding/Costs

- Staff (trial conduct/regulation)
- Database
- Drug/placebo
- Drug shipping
- Imaging
- Blood tests
- Insurance
- Pharmacy fees

PROMISE: Quoted to be > £150K!!!
Trial Challenges: Cutting Costs

- Drug – Sirolimus
  - Off patent
  - Pfizer provided drug free of charge
- Database
  - Excel workbook
- Insurance
  - 3 independent, identical trials
- Drug shipping
  - Separate contracts with Pfizer

- **Total cost for PROMISE in region of £60-70k**

- Speak with pharmaceutical partners
- Haggle with CTUs
- Simplify where possible
Conclusions

• Spectrum of conditions caused by mosaic mutations in PI3K-AKT signalling pathway
• PIK3CA most common
• Therapy with inhibitors of PI3K-AKT signalling pathway may be feasible
  – mTOR inhibitors
  – Selective p110α inhibitors
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- Veronica Kinsler

Referring physicians
The patients and their families
Any Questions?
PIP$_3$ Levels Everolimus Treated Cells

A.

B.