APPLICATION FOR A GOSHCC SURGICAL SCIENTIST PHD STUDENTSHP

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ICH Programme/Section: DBC

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1. Title
Investigating the function of IL1beta signalling in mouse and human paediatric craniopharyngioma

2. Portfolio summary

Aims:
Our current research has highlighted IL1beta signalling as a critical mediator of tumourigenesis in mouse and human adamantinomatous craniopharyngioma (ACP). The aim of the proposal is to reveal the function of IL1beta in ACP combining mouse genetics and molecular approaches with pre-clinical trials and small proof-of-principle studies in human patients with ACP.

Background:
ACPs are clinically important tumours due to tendency to invade the hypothalamus and visual pathways, leading to a high degree of morbidity and late onset mortality. ACPs are complex tumours comprising solid components and frequently large cysts filled with fluid rich in lipids and inflammatory mediators. So far, no specific targeted therapies have been developed, and current therapeutic modalities (i.e. surgery and radiotherapy) are often non-curative and associated with very high morbidity and tumour recurrence. There is an urgent clinical need to improve clinical care and outcome for these children 1.

The applicants have recently demonstrated the expression of multiple cytokines/chemokines in the solid cellular components of ACP tumours (Fig. 1) and cystic fluid (Fig. 2) and gene set enrichment analysis has shown a molecular signature in line with active inflammasomes (Fig. 3A). The inflammasomes are key signalling multiprotein complexes whose activation leads to the secretion of interleukin IL1beta and IL18, which promotes the establishment of an inflammatory microenvironment and the secretion of IL6, IL8 and TNFalpha from immune cells2. Our findings suggest that NLRP3 inflammasome activation may be mediated by the cholesterol crystals present in human ACP, as revealed by the accumulation of immune cells around the cholesterol clefts and a molecular signature similar to that obtained in atherosclerosis plaques (Fig. 4). Molecular profiling of mouse tumours has also demonstrated a similar signature and high expression of interleukins (Fig. 1A and Fig. 2B) (Manuscript under review in Acta Neuropathologica).

The IL1beta pathway is of particular interest for several reasons: (1) It is pro-tumourigenic by increasing proliferation and promoting malignancy in numerous tumours/cancers3; (2) Anakinra (an IL1 receptor antagonist that blocks IL1beta signalling), is routinely used in children against autoimmune disease4,5; (3) The safety profile and dosing schedules of Anakinra have been determined in children and therefore potentially offer a therapeutic option to allow rapid translation into the clinic if there is sufficient biological/ preclinical rationale for patients with a significant unmet need.
Proposed methodology to be adopted:

1. Mouse genetics studies to determine the effects of suppressing IL1beta signalling in the context of our ACP mouse models. Flox alleles are available from the Jackson Labs to disrupt IL1beta signalling genetically. Specifically, the student will use the IL1 receptor flox conditional allele to disrupt IL1beta signalling in different tumour compartments (Jackson Stock number 028398, Il1rloxP).

2. Preclinical studies in vitro and in vivo. The student will perform pre-clinical research by combining ex-vivo approaches with longitudinal MRI-embedded pre-clinical trials in vivo using our ACP mouse models. The student will test IL1beta inhibitors (e.g. Anakinra) and other inhibitors acting downstream (e.g. Etanercept, TNFalpha inhibitor). We will assess the overall survival and tumour burden in vehicle- and drug-treated mice. Additionally, we will investigate the molecular and cellular mechanisms underlying the observed effects.

3. Proof-of-principle clinical studies in human patients with ACP. ACP Patients with large cysts usually have a catheter implanted into the cyst to allow aspiration whenever the cyst grows. As Anakinra is a safe, well-tolerated drug commonly used in paediatrics, the student will perform studies to assess the effects caused by systemic administration of Anakinra on cystic refilling and cystic fluid cytokine profile. The effects of Anakinra will be monitored by quantifying cytokines known to act downstream of IL1beta (e.g. IL6, IL8, IL18, TNFalpha). In addition, the student will assess the cytokine profile of serum samples from ACP patients and healthy controls, to identify specific signatures. For instance, he/she will determine whether the natural IL1beta receptor antagonist (the product of the gene ILR1N) is elevated in ACP patients. Our collaborators within the Childhood Craniopharyngioma Research Consortium (CCRC) that JP leads will facilitate this research as we have samples from over 500 patients.

Skills to be achieved by the PhD trainee:

Mouse genetics, molecular approaches (e.g. qRT-PCR, RNA-Seq, ELISA), surgical approaches.

Relevance to the area of paediatric surgery:

ACPs are the most challenging suprasellar tumours in children. A radical surgical approach, aiming to remove the whole tumour, is problematic if there is invasion of the hypothalamus, as injury to this important CNS structure leads to unacceptable permanent morbidity and late onset mortality. A conservative surgical approach, with subtotal tumour resection, is preferred but this is associated with a high rate of tumour recurrence, need for further surgery and radiotherapy, as well as higher morbidity and mortality. In patients with large cysts, compression of surrounding structures causes significant symptoms and neural injury, particularly to the anterior visual pathways, and drainage only leads to transient improvement as these cysts usually refill.

This proposal may reveal a novel treatment against human ACP, by repurposing a well-known agent, that could prevent or delay tumour recurrence and/or cyst refilling. This will have rapid and important implications in reducing morbidity and mortality.

References:

A

Cytokine expression in mouse and human ACP (cellular components)

<table>
<thead>
<tr>
<th></th>
<th>IL1α</th>
<th>IL1β</th>
<th>IL18</th>
<th>IL6</th>
<th>IL8</th>
<th>TNFα</th>
<th>CCL3</th>
<th>CCL4</th>
<th>CCL5</th>
<th>CXCL5</th>
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<tbody>
<tr>
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<td>7.01</td>
<td>16.82</td>
<td>1.61</td>
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<td>10.41</td>
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<td>7.51</td>
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<tr>
<td>Murine ACP</td>
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<td>29.83</td>
<td>1.04</td>
<td>2.44</td>
<td>Ccl3: 1.15 Ccl4: 30.6 Ccl5: 29.65</td>
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<td>32.98</td>
<td>6.83</td>
<td>22.08</td>
<td>27.66</td>
<td>5.35</td>
</tr>
</tbody>
</table>

B

Inflammatory infiltrate in the solid component of human ACP

C

Correlation of expression of cytokines with CD14+ve immune cells

Figure 1. Expression of cytokines and chemokines in the cellular components of human and murine ACP is associated with an immune cell infiltrate. (A) Several interleukins, cytokines and chemokines are up-regulated in human and murine ACP. The Table includes only a subset of the up-regulated inflammatory mediators as an example. Numbers indicate fold change; >1.0 indicates up-regulation in the tumours. IL8 did not pass the quality control of the human RNA-seq data. In the mouse, IL8 orthologues are Cxcl1-3. (B) Presence of CD68 and CD3 immune cells in human ACP. (C) Expression of IL18, IL1B and IL10 co-related with CD14 expression.
A

Expression of cytokines in the cystic fluid of human ACP

![Cytokine expression graph]

B

Positive correlation of IL1β with IL6, IL8 and TNF expression in human cystic fluid with

![Correlation graphs]

Figure 2. Expression of cytokines and interleukins in the cystic fluid in ten human ACP samples. (A) The expression of the indicated cytokines was assessed using Meso Scale Discovery systems multiplex ELISA plates. (B) The expression of IL1β correlates with levels of IL6, IL8 and TNF, supporting the inflammasome signature revealed in the cellular ACP components.
**A**

**HUMAN ACP (cellular components)**

<table>
<thead>
<tr>
<th>Gene signature of macrophages treated with IL1</th>
<th>Gene signature of uterine muscle treated with IL1</th>
<th>Gene signature of chondrocytes treated with IL1</th>
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<tbody>
<tr>
<td>NES=1.79 FDR=0.001</td>
<td>NES=2.66 FDR=0.001</td>
<td>NES=2.25 FDR=0.001</td>
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</table>

**B**

**MURINE ACP (cellular components)**

<table>
<thead>
<tr>
<th>Gene signature of macrophages treated with IL1</th>
<th>Gene signature of uterine muscle treated with IL1</th>
<th>Gene signature of chondrocytes treated with IL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NES=1.38 FDR=0.02</td>
<td>NES=1.37 FDR=0.079</td>
<td>NES=1.39 FDR=0.075</td>
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</tbody>
</table>

**Figure 3.** The solid cellular components of human and murine ACP show a molecular signature of IL1 -induced inflammaGon. (A and B) Gene set enrichment analysis of human and murine ACP demonstrate an enrichment for genes that are up-regulated in cells treated with IL1.
A
CD68 immune cells and expression of CCL2 expression is associated with cholesterol clefts in human ACP

B
GSEA showing enrichment of atherosclerosis signature in human ACP

Figure 4. Evidence for a cholesterol-driven inflammasome response in human ACP. (A) CD68+ immune cells and expression of CCL2 is detected around the cholesterol clefts. A few cholesterol clefts are indicated with an asterisk (*). Cholesterol is dissolved during the histological processing of the samples. (B) Gene set enrichment analysis revealing a molecular signature in human ACP similar to that observed in atherosclerosis.