Research Review™
SPEAKER SERIES
2014 Tuberous Sclerosis Complex Australian Network Meeting

This publication is a summary of the Tuberous Sclerosis Complex (TSC) Australian Network Meeting held in Melbourne on October 18, 2014. This network meeting was convened to bring together the many clinicians nowadays involved in the management of TSC patients, and to further collaborative management and research in this area. It was highly successful in this regard, allowing dissemination of useful information and giving much useful practical advice for patient care. The meeting was chaired by Dr Sean Kennedy, paediatric nephrologist from Sydney Children's Hospital, with editorial oversight for this publication provided by A/Prof Deborah Yates, thoracic physician from St Vincent's Hospital, Sydney.

Associate Professor Deborah Yates

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A/Prof Yates is a Respiratory Physician in the Department of Thoracic Medicine at St Vincent's Hospital in Sydney and a Conjoint Associate Professor at the University of NSW. She has a longstanding clinical and research interest in obstructive lung disease, including asthma, COPD and lymphangioleiomyomatosis (LAM), and also in occupational lung disorders including asbestos-related disorders and occupational asthma. Her current research interests also include the development of non-invasive methods for assessing lung disease, such as the use of exhaled nitric oxide as a clinical tool for assessing asthma.

Abbreviations used in this Review:

AML = angiomyolipoma; **ESRF** = end-stage renal failure; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **LAM** = lymphangioleiomyomatosis; **LOH =** loss of heterozygosity; **mRCC** = metastatic renal cell carcinoma; **mTOR** = mammalian target of rapamycin; **PCKD** = polycystic kidney disease; **SEGAs** = subependymal giant cell astrocytomas; **TKI** = tyrosine kinase inhibitor; **TSC** = tuberous sclerosis complex

Introduction

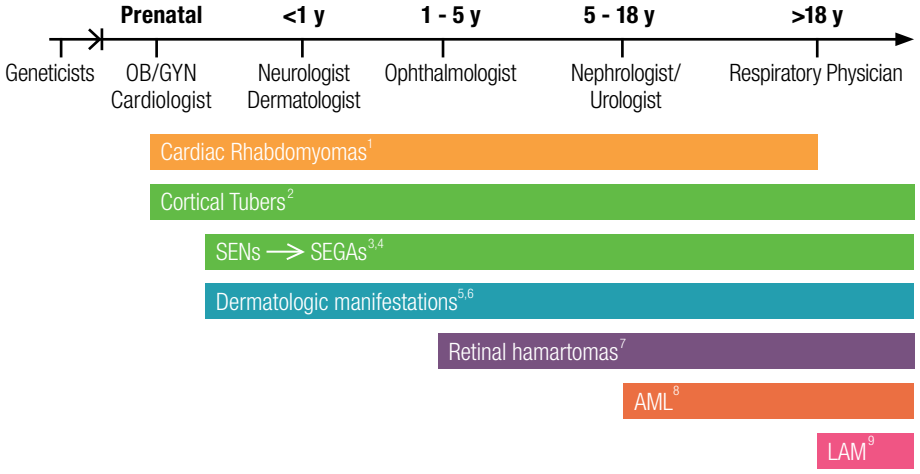
To highlight the multisystem involvement of TSC, Dr Kennedy gave the example of a child who over her lifetime would need the care of up to 14 different specialists, including neurologists, developmental paediatricians, geneticists, dermatologists, nephrologists and pulmonologists. How can clinicians provide optimal care for patients with TSC and connect with the other specialists that their patients may need? Tuberous Sclerosis (TS) Australia has played an integral role in optimising TSC care for Australian patients. TS Australia was formed in 1981 as The Australasian Tuberous Sclerosis Society (https://www.tsa.org.au/) and its activities include education, peer support, conference and social events, research funding, and advocacy. The society is an active member of TSC International and is developing the TSC Professionals Directory, an expansion of an existing patient nominated list. Dr Kennedy's questions for delegates to think about over the course of the meeting were:

- How do we ensure the best outcomes for Australians with TSC?
- How do we share experience and expertise for this rare disease?
- How do we develop and progress a research agenda?

Understanding TSC – diagnosis and general overview

Clinical geneticist, Dr David Mowat from Sydney Children's Hospital gave an overview of the diagnosis of TSC. The disease is an autosomal dominant genetic condition with an estimated worldwide incidence of 1 in 6000 and prevalence of 1 in 10,000–20,000. TSC affects around 1000+ people in Australia. Patients have variable degrees of seizures, intellectual disability and multisystem hamartomatous growth. Different organs are affected at different ages, which is important to keep in mind when diagnosing this disease (see Fig 1). For example, lymphangioleiomyomatosis (LAM) does not appear until adulthood, whereas cardiac rhabdomyomas are detectable in about 50% of foetuses with TSC but are quite benign and disappear over the first few years of life. It would be very useful to understand the biology of cardiac rhabdomyomas because a natural pathway already exists for resolution of these lesions.

Figure 1. **Manifestations of TSC**



AML, angiomyolipoma; **LAM**, lymphangioleiomyomatosis; **SEGA**, subependymal giant-cell astrocytoma; **SEN**, subependymal nodule

The TSC2 gene was identified in 1993 and the TSC1 gene in 1997. In 2003, the mTOR pathway was discovered, which is the main pathway involved in TSC. The different effects of various components of the mTOR pathway are still under investigation in TSC, but it is known that over activity in this pathway is involved in the development of tumours, diabetes, obesity, epilepsy and autism. The mTOR inhibitors everolimus and sirolimus act by blocking this pathway. The genetics of TSC are very simple. The disease is autosomal dominant, near 100% penetrance with variable expressivity, with two thirds de novo and one third familial. About 15–20% of patients have mutations in the TSC1 gene (less severe disease), 60–70% have mutations in the TSC2 gene (more severe disease) and 10–15% of patients have no mutation identified. Patients with mutations in TSC2 are more likely to have intellectual disability, seizures, subependymal nodules, angiomyolipomas (AMLs) and renal cysts. It is likely that there are other genetic mutations involved in TSC.

In 2006, sirolimus was used effectively for the first time in patients with subependymal giant cell astrocytomas (SEGAs) associated with TSC. Everolimus was approved by the FDA (US) in 2010 for the treatment of SEGAs associated with TSC and in 2012 for AML associated with TSC. In Australia, everolimus was listed on the PBS in December 2013 for TSC as follows: SEGAs associated with TSC or visceral tumours (LAM, AMLs) associated with TSC and the treatment must be the sole PBS-subsidized therapy for this condition and the patient must not be a candidate for curative surgical resection.

In 2012, the International TSC Consensus Conference published updated guidelines for diagnostic criteria[10] and management[11] of TSC. The main difference from the previous guidelines is that diagnostic guidelines now include a sole genetic diagnostic criteria, i.e. patients may be diagnosed with TSC if they have a pathogenic TSC1 or TSC2 mutation.

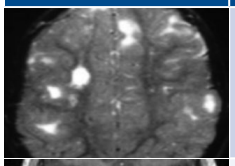
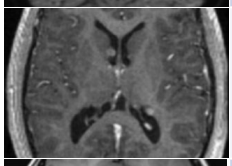
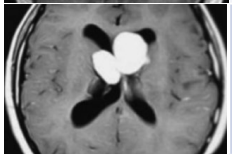
Current research underway in TSC includes:

- Biomarkers in TSC and autism
- Randomised controlled trials in epilepsy (EXIST-3 trial) and autism
- Sirolimus and autophagy in LAM
- Next generation sequencing in patients with no mutation identified

Management of CNS manifestations in TSC

Dr John Lawson from Sydney Children's Hospital focused on management of CNS manifestations in TSC including SEGAs, epilepsy, and behavioural and cognitive disorders. The main brain lesions involved in TSC are cortical tubers, subependymal nodules and SEGAs (see Fig 2).

Figure 2. **Lesions of the brain in TSC**

	Type	Incidence	Features
	**Cortical tubers**	**95%-100%**	• Focal abnormality of cortical architecture with loss of the typical 6-layered structure of the cerebral cortex • May contribute to epileptogenesis
	**Subependymal nodules**	**88%-98%**	• Growth on subependymal lining of the lateral ventricles; typically considered unrelatable to neurologic symptoms
	**Subependymal giant-cell astrocytomas**	**6%-19%**	• Near the Monro foramina • May grow and block cerebrospinal fluid through the lateral ventricles, leading to hydrocephalus

Subependymal Giant-cell Astrocytomas (SEGAs)

There is no consensus on the definition of SEGAs. The EXIST-1 study defined SEGAs as ≥1.0 cm in longest diameter and serial growth of at least 25%[12] and a European study defined SEGAs as a markedly enhancing lesion near the foramen of Monro.[13] SEGAs are bilateral in one third of patients and there are no comprehensive data on their natural history. The average age of SEGA presentation is 11 years (rare >20 years) and its prevalence in TSC is 5–20%. SEGA patients are usually asymptomatic and thus are diagnosed on CT or MRI with sonography useful in neonates. In fact, SEGAs are increasingly being diagnosed antenatally. If patients are symptomatic, they often have signs of raised intracranial pressure.

Treatment options for SEGAs include watch and wait, surgical resection, medical therapy or other interventions such as radio-surgery, laser or shunt. Factors in treatment decision include:

- Parental/patient preference
- Size and rate of growth
- Location of lesion/neurosurgical access
- Severity of symptoms
- Tolerability of pharmacotherapy
- Consequences of surgery
- Surgical experience
- Multiplicity of TSC lesions
- TSC co-morbidities

Watching and waiting is the recommended approach for an asymptomatic lesion which is not growing. Serial neuroimaging is recommended every 1 to 3 years to monitor SEGA progression in asymptomatic patients. This routine monitoring approach can identify a SEGA with potential for growth and delay traditional surgical intervention by allowing the choice of medical therapy. Untreated SEGA is associated with chronic hydrocephalus or acute hydrocephalus and death with bleeding and mortality of about 50%.[14]

There are no randomised controlled trial data on the consequences of surgery in patients with SEGAs so it is difficult to make a decision in this regard. Surgery is the treatment of choice for patients presenting with symptomatic hydrocephalus. In a small surgical trial 18 patients with TSC underwent 22 primary tumour resections for SEGAs. Thirty-nine percent required a ventriculoperitoneal shunt post-surgery and 2 needed repeat surgery after average follow-up duration of 52 months.[15] Other small surgical series have also demonstrated the risks associated with surgery: recurrence risk if surgery is incomplete (about 25% of patients will need repeat surgery); ventriculoperitoneal shunts required in 20–50%; hemiparesis in 0–5%; and mortality of 0–10%.

In a pioneering study of sirolimus in five TSC patients with SEGAs a marked reduction in tumour volume was seen at 3–6 months.[16] The same centre conducted a phase II trial of everolimus in 28 TSC patients with SEGAs.[17] After long term follow-up of 4–5 years all patients had a reduction from baseline in tumour volume, including about one third of patients with a 50% reduction. The double blind randomised controlled EXIST-1 trial of everolimus in 117 patients with TSC-associated SEGAs showed a 50% reduction in tumour size at 6 months in one third of patients.[12] Similar efficacy was demonstrated in an Australian case series of 13 children and adolescents treated with everolimus or sirolimus.[18]

The 2012 International TSC guidelines for management of SEGAs[19] recommends:

- Screening MRI every 1–3 years until 25 years old
- Medical vs surgical treatment decision to be made by each individual physician
- Surgery recommended in acute life threatening SEGAs
- Medical therapy recommended for locally invasive, bilateral, or large tumours
- TSC co-morbidity may favour medical vs surgical treatment
- Parental preference for medical vs surgical treatment must be taken into account

Epilepsy

Epilepsy is very common in TSC. A retrospective review by Chu-Shore et al. of 291 TSC patients showed that 85% had epilepsy with a median age of onset of 7 months.[20] One third of these patients had infantile spasms. Two thirds had refractory epilepsy and half had more than one seizure type. Among 39 patients who underwent surgery, only 10 became seizure free. IQ was <70 in 60% of patients with seizures and 12% of patients with no history of seizures.

Therapeutic options in severe childhood epilepsy include surgery, antiepileptic drugs, ketogenic diet and vagus nerve stimulation. In a systematic review of 177 TSC patients undergoing surgery for epilepsy, 57% were seizure-free at 2 years and an additional 18% had >90% improvement in seizures.[21] Factors associated with higher surgical failure rate included presence of tonic seizures and IQ <70. In a Mayo Clinic study 22 patients with TSC underwent epilepsy surgery and were followed for up to 14 years.[22] Twenty-three percent of patients who were seizure free at 1 year had relapsed by 5 years.

Around 10% of children with infantile spasms have TSC. A review of 10 studies of vigabatrin for infantile spasms showed that 170 of 313 (54%) patients without TSC became spasm free versus 73 of 77 (95%) patients with TSC. About 25% of patients developed subsequent partial seizures. Broadly speaking, spasm control leads to improved development. However, vigabatrin has been associated with a 30–50% incidence of visual field defects according to epilepsy clinical studies of around 10 years follow-up, which is cumulative dose-dependent.[23,24]

The effect of everolimus on seizure control was assessed in a prospective, multicentre, open-label, phase I/II clinical trial of patients with TSC and refractory epilepsy.[25] Among 23 enrolled patients, median age was 8 years, median seizure frequency was 1 per day and patients had a median of 2 previous antiepileptic drugs. At 12 weeks, seizure frequency was reduced by ≥50% in 12 of 20 treated patients. Overall, seizures were reduced in 17 of the 20 patients by a median of 73% ($p<0.001$). Seizure frequency was also reduced during 23-hour EEG monitoring ($p=0.007$). Significant reductions in seizure duration and improvement in parent-reported behaviour and quality of life were also observed. The success of that trial led to EXIST-3, a double-blind, randomised controlled trial evaluating the efficacy and safety of two trough-ranges of everolimus given as adjunctive therapy in patients with TSC who have refractory partial-onset seizures. The study aims to recruit 355 patients internationally; 4 Australian sites are enrolling patients (NCT01713946).

Behavioural and cognitive disorders

Behavioural and cognitive disorders are often the largest burdens for patients with TSC and their families. According to historical data, around 30% of patients with TSC have profound intellectual disability (IQ <20%). Classic autism is seen in 25%, autism spectrum disorder in 25–50%, ADHD in 50% and anxiety disorders in 60%. Is it possible to shift patients out of this low IQ group into a higher range, and if so, how is this achieved?

There is an altered neuronal connectivity in TSC – does this contribute to neurological deficits? There is no loss of heterozygosity (LOH) in these neurones, but they are abnormal. Dendritic spines are submicron membranous protrusions where >90% of excitatory synapses terminate. TSC studies in humans and rats show fewer spines and altered shape. This is a potential basis for autism and intellectual disability in TSC, but has not been proven relevant yet. Studies of sirolimus in animal models of TSC and cognitive disorders look promising. Reversal of learning deficits were seen in TSC2 (+/–) mice with TSC given sirolimus.[26] The therapeutic value of prenatal sirolimus was shown in a mouse brain model of TSC.[27] Pulse sirolimus therapy was effective in a rat model of infantile spasms and associated cognitive decline.[28]

Management of TSC-associated renal AML: a urologist's perspective

Dr Simon Wood from Princess Alexandra Hospital stated that the key aim in managing the renal manifestations in TSC is to identify and treat patients with high risk lesions that are likely to progress and develop symptoms. The other priority is to preserve renal function by avoiding unnecessary intervention, primarily multiple embolisations or surgery. The challenge for clinicians is to work out which lesions are high risk and deserve treatment. The ability to do this at the moment is rather limited, primarily because the natural history of this disease is not entirely understood. Knowledge today is based on historical cohorts and opinion.

Unregulated increase in mTOR signaling in the kidney produces cellular proliferation, angiogenesis and increased cell survival. AMLs are the most common renal manifestation of TSC, affecting 50–80% of patients, and are often multiple and bilateral. The pathogenesis of these lesions appears to involve aberrant differentiation of progenitor cells.[29] AMLs contain varying proportions of abnormal vessels, fat and immature smooth muscle cells.[29] Some lesions are fat-poor which can create a diagnostic dilemma. The aberrant vasculature has a propensity to form intratumoural aneurysms and arteriovenous malformations which confers a risk of haemorrhage. There are a number of epithelial lesions that occur in addition to AMLs. The most common are renal cysts (incidence 30% and usually of no significance), polycystic kidney disease (PCKD) with contiguous loss of TSC2/PKD1 (1%) and renal carcinoma (2–4%).[30]

In terms of the natural history of renal AMLs in TSC, they are different to sporadic AMLs in that they typically appear at a much earlier age and are larger, multifocal and bilateral. AML growth rate is usually highest in childhood and adolescence; ~40% of patients have a very high renal tumor burden[31]; PCKD rates are higher than the general population[31]; end-stage renal failure (ESRF) is rare[32] and when it does occur is usually a result of interventions, i.e. multiple embolisations or surgery, or occasionally PCKD phenotype; ANZDATA report an annual incidence of ESRF in TSC of <1 (0.03%) – this appears to be much lower than other parts of the world, particularly the US where there is a considerably higher intervention rate in AMLs.

The main issues to consider when determining the need for intervention in renal AMLs are: size of the lesion, the growth of the lesion and the presence of vascular abnormalities. The most commonly cited paper referring to cause of death in TSC is a Mayo Clinic publication from 1991 that states 'Renal disease is the most common cause of death in Tuberous sclerosis patients'.[33] The implication of this statement is that these are dangerous lesions that deserve excision or intervention, which has driven a very high intervention rate. In fact only 2 of 40 deaths in the study were a result of renal bleeding. SEGAs, epilepsy and LAM were more likely to result in death. The obvious risk is that in the current era of increased screening detecting incidental, asymptomatic lesions we risk over treatment! A longitudinal series of 54 patients with TSC-associated AMLs showed the risk of bleeding for patients with a growing lesion was 1 in 3.[31] In contrast, the risk of bleeding in a stable lesion was 1 in 10. Of note, bleeding was not radiologically defined. Importantly, 70% of patients with AMLs had no symptoms or complications. Regarding management options for TSC-associated renal AMLs, most patients remain asymptomatic. The best approach for these patients is active surveillance every 1–3 years. If there is a trigger for treatment, therapy with an mTOR inhibitor is first-line. Selective angioembolisation is effective for controlling haemorrhage[10] but has limited long-term value as repeat procedures may be required.

Dr Wood stressed that the key message is that surgery has a very limited role in the management of renal lesions. Regrettably, nephrectomy continues to be performed as treatment for large AMLs. The important message for urologists is that just because a kidney is large and disorganized and replaced by large AMLs and appears worrisome and untreatable, that patient is not well served by having a nephrectomy. Unfortunately, most series in the literature have significant rates of nephrectomy for this indication. Where excision of a lesion is required, e.g. an indeterminate lesion suspicious for cancer or a confirmed cancer, or occasionally an elderly patient with an isolated exophytic lesion with high risk features, then nephrectomy can be a good treatment. But the call for such surgery is very rare.

Case report

Dr Wood discussed a case report from his clinic. A 37-year-old female patient had a small indeterminate lesion in her right kidney and small pulmonary nodules. The presumed diagnosis was metastatic renal cell carcinoma (mRCC). The patient had a right nephrectomy and treatment with a tyrosine kinase inhibitor (TKI) was initiated. After one year of drug therapy, the patient had no change in her pulmonary nodules and TKI therapy was discontinued due to adverse effects. A number of years later (in 2013) the patient was referred to Dr Wood's clinic with a solid lesion in her solitary left kidney. Her pulmonary lesions were diagnosed as a benign manifestation of TSC (multifocal pneumocyte hyperplasia). A partial nephrectomy was performed and the patient has ultimately done well.

Summary

- Many patients with TSC-associated AMLs do not suffer symptoms or complications and can be managed with active surveillance
- There are higher risk lesions and triggers for intervention with systemic therapy
- Angioembolisation is good for salvage of symptomatic or bleeding lesions but should not be routinely employed for all large lesions
- Nephrectomy is almost never indicated for TSC-associated AMLs

Management of TSC-associated renal AML: a nephrologists perspective

A/Prof Nikky Isbel from Princess Alexandra Hospital said it has been difficult to get an adult TSC clinic established in Australia. This is probably because patients have an established care pathway from when they are diagnosed as children and the transition to adult services is difficult. Some patients do present as adults with no prior diagnosis which has been a major learning curve for A/Prof Isbel and her team. In a retrospective review from the NIH, 79 patients were diagnosed with TSC as adults.[34] Sixty-six percent had symptoms suggesting the diagnosis was missed in childhood, most had mild skin manifestations, 90% had AML, 81% had LAM, 27% had epilepsy and 27% had a positive family history of TSC. When patients present with the disease as adults they often have more subtle features but this a multisystem disorder - clinicians need to look! Recommendations from the consensus statement[11] recommend the following examinations:

- Kidneys – MRI abdomen
- Lungs – renal function test and HRCT lungs in women >18 years
- Brain – MRI +/– EEG as appropriate
- Dermatological
- Ophthalmological
- Cardiac assessment – ECG, echo if concern
- Neuropsychiatric assessment

TSC is a very significant diagnosis for patients and their families and management can be challenging. Intellectual impairment is evident in ~50% patients with TSC and it is difficult ensuring that adult patients understand instructions regarding blood tests, adverse effects, etc. Anxiety is also very common in TSC patients; explaining the diagnosis and potential adverse effect profile of medications can be intense. There are also implications for existing first degree relatives and future fertility.

A multidisciplinary team is still evolving at Princess Alexandra Hospital. The group is made up of a urologist, nephrologist, radiologist, an off-site respiratory physician with expertise in LAM and a paediatric team. Patients generally already have their own neurologist, and dermatologists and geneticists at Royal Brisbane Hospital are available. The team does not yet have a dedicated psychiatrist/psychologist.

Treatment of renal AML with mTOR inhibitors

There are randomised but small trials showing efficacy of mTOR inhibitors in renal AML using a surrogate endpoint. It is important to remember that there is no hard clinical patient-relevant endpoint for renal manifestations in TSC because the event rate of significant bleeding and progression of renal disease is very rare and slow. It would be extraordinarily difficult to set up a trial using these endpoints.

EXIST-2 was a double-blind, randomised controlled phase III study of everolimus for the treatment of AML in adult patients with either TSC or sporadic LAM.[35] The primary endpoint was the proportion of patients with an AML response of at least a 50% reduction in total volume of target AMLs relative to baseline. 118 patients (median age 31 years; IQ 18–61) from 24 centres in 11 countries were randomly assigned to receive everolimus 10 mg/day (n=79) or placebo (n=39). At data cut-off, double-blind treatment was ongoing for 98 patients. The AML response rate was 41.8% for everolimus and 0% for placebo (see Table 1). The difference in response rate was 41.8%. The most common adverse events in the everolimus and placebo groups were stomatitis (48% and 8%, respectively), nasopharyngitis (24% and 31%), and acne-like skin lesions (22% and 5%).

Table 1. **Outcomes in EXIST-2**

Response, n (%)	Everolimus n = 79	Placebo n = 39
Primary analysis		
AML response rate [95% CI]	33 (41.8) [30.8, 53.4]	0 [0.0, 9.0]
P-value	< 0.0001	
Difference % (95% CI)	41.8 (23.5, 58.4)	
Best overall AML response		
Response	33 (41.8)	0
Stable disease	32 (40.5)	31 (79.5)
Progression	1 (1.3)	2 (5.1)
Not evaluable	13 (16.5)	6 (15.4)

In the EXIST-1 SEGA trial, subgroup analysis showed that by 48 weeks, 80% of patients had >50% reduction in renal AMLs.[36] Sirolimus has also been reported to be effective in renal manifestations of TSC. In an open-label trial of 25 patients with TSC and AML or LAM, AML regressed somewhat during 12 months of sirolimus therapy but tended to increase in volume after sirolimus was stopped.[37]

Pharmacokinetics studies of everolimus show that it is rapidly absorbed with a T_{max} in healthy individuals of 30–60 min. TSC patients receive much higher doses of the drug compared with transplant recipients. Everolimus absorption is decreased by fatty food; C_{max} decreases by 60% and AUC decreases by 16%. The drug undergoes hepatic metabolism and has a half-life of 30 hours. It is a substrate of CYP3A4 and P-glycoprotein and drug interactions are particularly relevant (i.e. phenytoin, carbamazepine). In EXIST-2, the median target C_{min} range was 5–15 ng/mL and median everolimus dose was 8.6 mg/day. Dose reduction or interruption was required by 48% in the everolimus group versus 21% in the placebo group. Mean everolimus trough levels showed large inter-individual variability. Patients had a 10% tumour size reduction from baseline for a 2-fold C_{min} increase (95% CI –16% to –4%). A/Prof Isbel's group has found it difficult for their patients to achieve the same target C_{min} range required in EXIST-2 due to adverse effects. Her group conducted an observational study of 4 patients with TSC and large renal AMLs. Patients were aged 16–40 years, had >10 lesions and bilateral involvement. Most had been referred for consideration of nephrectomy or major surgery. Patients commenced on everolimus 10 mg/day and were followed for a median of 12 months. The mean everolimus trough level at 12 months was 2.9 ng/mL, and of note, no patient achieved the recommended EXIST-2 target level of 5–15 ng/mL. Adverse effects included mouth ulcers/stomatitis (n=4), interstitial pneumonitis (n=1) and severe migraine (n=2). Two patients required dose reduction (interstitial pneumonitis, severe mouth ulcers), 1 required dose increase (due to dilantin interaction) and 1 remained on 10 mg/day (temporary cessation for inguinal hernia repair). All target lesions reduced in size by 18–51%. It is unknown if C_{min} is critical to lesion regression or if it is possible to achieve the same outcome with lower doses over a longer period of time.

A/Prof Isbel's current protocol is to initiate everolimus at 10 mg/day if patients are on an enzyme-inducing antiepileptic drug and at 5 mg/day if patients are not on an enzyme-inducing antiepileptic drug. The drug is titrated to efficacy and adverse effects and drug holidays used if required.

A/Prof Isbel presented two case studies from Princess Alexandra Hospital. The first was a 39-year-old female with multiple large bilateral renal AMLs. She had occasional major haemorrhages requiring hospitalization (~ yearly). She had undergone multiple angioembolisations and transfusions and embolisation was no longer a safe option. The patient was referred for bilateral nephrectomy and live donor transplant from her mother. Her creatinine was 100. The patient had no epilepsy or cognitive impairment but suffered from severe migraines and anxiety. A diagnosis of TSC was made at a geneticist's clinic. The patient received everolimus under a compassionate use programme with good initial effect and reduction in renal AML volume. She then presented with shortness of breath, cough and interstitial pneumonitis. HRCT scan revealed LAM. She has been referred for further review. The second case was a 16-year-old female with bilateral AMLs including a large right renal AML that had extended into the renal vein and inferior vena cava. The patient had undergone previous embolisation, and was referred for nephrectomy and inferior vena cava resection. She had multiple hamartomas (including cardiac), epilepsy, minor intellectual disability, depression, reduced vision in her right eye, and facial angiofibromas. She had a very good response to everolimus therapy.

Summary

- Everolimus is a promising therapy for patients with large or multifocal AMLs
- May avoid treatments that threaten normal surrounding renal parenchyma
- Major surgery was avoided in all patients
- Clear cosmetic benefit
- Significant adverse effects can occur and patients need to be carefully monitored
- Unanswered questions remain in terms of drug dosing, drug targeting and long-term maintenance therapy

Management of LAM in TSC

A/Prof Deborah Yates from St Vincent's Hospital Sydney discussed the management of LAM in TSC. The spectrum of lung disease in TSC includes pneumothorax, LAM and chylothorax, multifocal micro nodular pneumocyte hyperplasia, and asthma as well as all the usual lung diseases. LAM is a rare genetic systemic disease which almost exclusively affects women of child-bearing age. Lungs are destroyed by accumulation of unusual smooth muscle cells (LAM cells) producing widespread thin walled cysts. These cells stain positive for smooth muscle, HMB-45, oestrogen/progesterone receptors, and beta catenin. The mechanism of LAM formation is still uncertain.

About 30–40% of women with TSC have LAM but it also occurs in the absence of TSC – known as sporadic LAM (sLAM). LAM is often initially misdiagnosed as asthma (bronchodilator responsiveness in up to 50%) and has recently been re-classified as one of the PEComas (perivascular epithelial cell tumours). Patients present with pneumothorax, breathlessness, haemoptysis or chyloptysis, or chylous pleural effusion, and sometimes with an abdominal mass or chylous ascites, and as breathlessness or pneumothorax in pregnancy. The rate of decline in FEV_1 is reported between 75–118 mL/year. The 10 year mortality rate is 10–20% from onset of symptoms, and 30% from time of lung biopsy.

Patients with chylous pleural effusion do not have malignant cells but if examined carefully LAM cells can be found. Abdominal LAMs are large fluctuant masses which appear retroperitoneally and are difficult to diagnose. They change throughout the day, probably due to obstruction of lymphatics by LAM cells. Abdominal LAMs are rare but respond well to mTOR inhibitors. Approximately 2% of all pleural effusions are due to chylothorax; chylothorax occurs in about 30% of women with LAM. Protein concentration is usually >3 g/dL, giving a milky appearance on thoracentesis. Absence of a milky appearance does not exclude chylothorax, especially if a patient is fasting.

In diagnosing LAM, one needs a high index of suspicion. The 2010 European Respiratory Society guidelines for the diagnosis of definite LAM[38] state:

- Characteristic or compatible: a lung HRCT, and lung biopsy fitting the pathological criteria for LAM. Or:
- Characteristic lung HRCT and any of the following:
  - AML (kidney)
  - thoracic or abdominal chylous effusion
  - LAM or lymph-node involved by LAM
  - definite or probable TSC

Management

Management of LAM should include: advice to avoid smoking, avoid oestrogen-containing treatments, maintain normal weight; warn of risks of air travel (pneumothorax); advice regarding pregnancy; regular influenza vaccination and pulmonary rehabilitation; assessment and management of osteoporosis, especially post-menopause; psychiatric review and support where necessary; cross-specialty referral where needed e.g. renal, dermatology, neurology.

Medical therapy of LAM is poorly supported by evidence. Historically, patients have been treated with inhaled bronchodilators and doxycycline. Treatment also included progesterone, oophorectomy, tamoxifen, and GnRH agonists, however these are not regularly recommended now. Progesterone can be trialled in patients with rapid decline for one year with monitoring of symptoms and lung function. Anti-oestrogen interventions are not currently recommended. The mTOR inhibitors sirolimus and everolimus are the most effective treatments to date. Trials are underway of simvastatin, chloroquine and saracatinib.

Two double-blind placebo-controlled trials of doxycycline showed no significant effect on any outcome measures in patients with LAM. (Chang et al. 2014[39]; Yates et al. unpublished). A number of clinical trials and case series have shown sirolimus[37,40-43] and everolimus[35,44] to be effective in the treatment of LAM. Only two of these studies were randomised controlled trials; results of these are shown in Table 2.

Table 2. **Randomised controlled trials of mTOR inhibition in LAM**

Treatment	Study design	Study details	Outcome
Sirolimus (MILES Trial)[40]	Double blind placebo controlled parallel group; 12 months treatment and 12 months observation	89 patients with LAM; primary end-point difference in FEV_1	Highly significant change in FEV slope (–12.2 mL/month in placebo; +1–2 mL/month with sirolimus treatment), improved quality of life, decreased VEGF-D. No change in DLCO or 6 min walk
Everolimus (EXIST-2 Trial)[35]	Double blind placebo controlled phase 3 study	118 patients with TSC or sporadic LAM	Reduction of AML volume

About 1.1% of LAM patients are lung transplant recipients. Survival is slightly better for such patients than for LAM patients with other conditions – 86% at 1 year, 76% at 3 years, and 65% at 5 years. Recurrence of LAM in the lungs is rare and occurs in around 7% of transplanted lungs, but does not seem to affect outcome. Bronchiolitis obliterans syndrome is the most frequent cause of death in these patients. One of the difficulties of classifying LAM as a malignancy is that patients would then in theory not be eligible for lung transplantation.

A specialised LAM clinic is now well established at St Vincent's Hospital. It is free of charge and open to all patients with potential LAM or similar diseases. Interstate referrals are welcome. The clinic has a standardised approach to data collection and treatment including quantitative HRCT scanning and exhaled breath analysis and has a close liaison with transplantation services and thoracic surgeons. It has strong links with the TSC clinic at Sydney Children's Hospital and with LAM support groups and cross refers to appropriate specialists (renal, neurology, surgical) and interstate. Patients receive everolimus on an individual patient basis with drug committee approval.

Summary

- Rapid developments in LAM are facilitated by international and national collaborative registries and patient groups
- mTOR inhibitor therapy shows real promise for prevention of progression in lung disease and in lymphatic involvement in LAM
- Australasian collaboration in international multicentre trials would be optimal

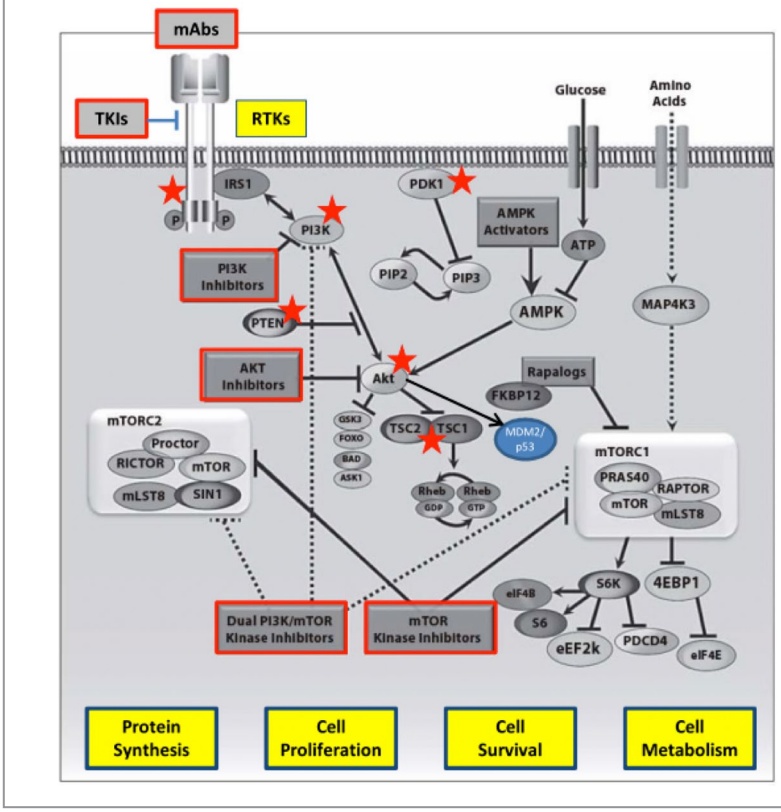
Management of dermatological manifestations in TSC

Dr Mowat explained that TSC manifests as facial angiofibromas in the skin with sun exposure causing acquired mutation. The molecular weight of sirolimus allows for its absorption through the superficial layers of the epidermis. With the appropriate delivery system, a topically applied formulation of sirolimus should be able to penetrate the superficial dermal layers to treat cutaneous facial angiofibromas. A number of small studies have shown that topical sirolimus significantly improves facial angiofibroma lesions in patients with TSC.[45-50] The TREATMENT Trial of sirolimus in facial angiofibromas is currently underway at 10 clinical sites (9 in the US and 1 in Australia). Enrollment has closed with a total of 174 subjects. Patients applied either placebo, low dose sirolimus (0.1%) or high dose sirolimus (1.0%) nightly and were followed for 6 months. Data has been analyzed for the first 50 subjects. There was a statistically significant improvement noted in patients on high and low dose sirolimus versus those on placebo (61% and 47% vs 5%; p=0.003). Itching, burning, and increased acne were reported in the sirolimus groups but there were no serious adverse events related to the drug. Sirolimus levels were undetectable in all subjects for the duration of the study. In Australia, multiple pharmacies are producing topical sirolimus, but there is large variability in quality. Dr Mowat's group is seeking a local manufacturer using the same preparation as used in the TREATMENT Trial; licensing issues are currently being addressed. The current cost for topical sirolimus 45g is US$195 (0.1%) and US$295 (1.0%).

mTOR inhibitors: from signalling pathway to pharmacotherapy

Dr Elgene Lim from Austin Hospital discussed the biology of the mTOR pathway from an oncologist's perspective. The mTOR pathway is part of the larger PI3K pathway (see Fig 3). One of the key differences in oncology is that mutations in this pathway are somatic, not germline. The stars in the figure represent sites of somatic mutation that occur in various types of cancer. PI3K is an important pathway in oncology which results in a lot of cell proliferation, but clearly other aspects such as cell survival and metabolism and protein synthesis are also downstream of this particular pathway. Besides mTOR, there are other multiple parts of the PI3K pathway which are targetable. Currently the only drugs reimbursable on the PBS are mTOR inhibitors but there are a slew of other drugs targeting parts of the pathway upstream, including EGFR receptor inhibitors, trastuzumab, TKIs, PI3K inhibitors, AKT inhibitors, and dual PI3K/mTOR inhibitors. However, if one part of the pathway is targeted, often there are compensatory mechanisms that kick in. The tumour is often smarter than we are!

Figure 3. **Multiple aberrations lead to PI3K/mTOR pathway activation**[51]



Dr Lim discussed a number of studies of everolimus in breast cancer and mRCC. In a phase II study, tamoxifen plus everolimus increased clinical benefit rate, time to progression, and overall survival compared with tamoxifen alone in postmenopausal women with aromatase-resistant metastatic breast cancer.[52] In the phase II BOLERO-2 study, everolimus combined with exemestane improved progression-free survival in patients with hormone receptor-positive advanced breast cancer previously treated with nonsteroidal aromatase inhibitors.[53] Everolimus plus exemestane extended overall survival, a secondary endpoint, by 4.4 months compared to exemestane alone, not reaching statistical significance.[54] The phase III BOLERO-3 study is underway to evaluate the addition of everolimus to trastuzumab plus vinorelbine.[55] Statistical significance was not reached at the interim overall survival analysis; final overall survival analysis will be conducted after 384 deaths.

In mRCC, everolimus monotherapy was well tolerated and showed potential benefits in phase I/II studies. The RECORD-1 phase III study enrolled pretreated mRCC patients with progression on VEGFR TKI. Everolimus treatment resulted in a 67% relative risk reduction in disease progression and more than doubled progression-free survival compared to placebo.[56] In the RECORD-3 phase II study non-inferiority of progression-free survival for first-line everolimus versus sunitinib was not achieved.[57] The safety profile for everolimus and sunitinib was consistent with prior experience. The trial concluded that the standard treatment paradigm remains first-line sunitinib followed by everolimus upon progression.

Understanding mTOR inhibitor adverse effects

Dr Kennedy discussed the management of TSC patients with respect to adverse effects associated with mTOR inhibitors. These drugs are used in a number of diseases because of their immunosuppressive, antiproliferative, and antiangiogenic effects. Therefore, it is not surprising that normal cell growth is compromised due to these properties, leading to various adverse effects.

The largest body of evidence available for mTOR inhibitors is in the renal transplant setting. Among such patients, 30% discontinued clinical trials because of adverse events. mTOR inhibitors were associated with increased wound complications if started early, infective and haematologic adverse effects when used in combination with other immunosuppressants, and proteinuria, particularly if used in kidneys with existing injury. In three short-term trials of everolimus in TSC, the main adverse effects associated with everolimus were stomatitis and mouth ulcers (all grades, 31–79%).[12,17,35] Acne-like lesions and hypercholesterolaemia were also experienced by more everolimus than placebo patients (22% vs 5% and 20% vs 3%, respectively).[35] Of note, the majority of these events were grade 1–2. In longer term trials (up to 4 years), the incidence of adverse effects decreased over time and no new events emerged.[12,35] This was most likely due to dose reduction and effective management.

In Dr Kennedy's experience, the likelihood of adverse effects generally increases with increasing plasma drug levels, showing the importance of therapeutic drug monitoring. Adverse effects that are particularly dose related include stomatitis, mouth ulceration, pneumonitis, dyslipidaemia and immunosuppression. Many adverse events are idiosyncratic and their timing is unpredictable. There are a number of clinically relevant drug interactions that clinicians should be aware of.

Adverse effects in the paediatric setting

The long-term effects of mTOR inhibitors in children are unknown. Clinicians should ensure immunisations are up to date before starting everolimus. Live vaccines should be avoided while receiving everolimus and zoster immunoglobulin considered if the patient has been exposed to varicella and is non-immune. It is worth considering drug holidays to update immunisations. Everolimus should be withheld during any serious infection. Initial reports from transplant literature raised the possibility that sirolimus was associated with impaired growth during childhood,[58] however, subsequent studies have not supported this finding.[59,60] Regarding fertility, secondary, transient amenorrhoea has been reported in everolimus trials. Adult males treated with mTOR inhibitors after kidney transplant have lower fertility than males on other regimens, including reduced sperm count, decreased proportion of motile spermatozoa and lower testosterone levels.[61-63] Reversibility of infertility is uncertain and clinicians should consider sperm bank for adolescent and adult males before initiation of therapy.[61-63]

Adverse effects in the adult setting

A/Prof Isbel discussed her experience of the adverse effects associated with mTOR inhibitors in adult TSC patients. Stomatitis, the most common dose limiting toxicity, has a median time to onset of 39.5 days. It may relate to a direct toxic effect on mucosa and presents as a distinct, painful ovoid ulcer surrounded by an erythematous margin on the inner lip, ventral and lateral surface of the tongue, or soft palate. Severe cases may be associated with erythema, oedema, burning sensation and occasional bleeding. Stomatitis can cause pain and limit oral intake and affect quality of life. Management includes good oral hygiene; avoidance of alcohol based mouth washes; and use of bicarbonate mouth wash, topical anaesthetic agents or topical corticosteroids. Stomatitis may settle with time but dose reduction or temporary cessation may be required.

Infections are also seen in many TSC patients. The most common are URTIs, sinusitis, and otitis media, however the majority are self-limiting. Infection risk should be assessed at baseline and patients tested for hepatitis B virus (HBV), HCV and HIV. Everolimus has been associated with HBV reactivation. In one study, 18% of patients with hepatocellular carcinoma who were HBV+ at baseline reported reactivation. One fatal case of HBV reactivation was reported in a patient with metastatic breast cancer receiving everolimus.[64] Patients should be advised to be aware of signs and symptoms of infections and to seek advice early.

Symptoms of non-infectious pneumonitis include dry cough, exertional dyspnoea and hypoxia, particularly on 6 minute walk. One case of non-infectious pneumonitis was seen in both EXIST- 1 and 2. Both were grade 2 and resolved with dose reduction. There are a number of theories on the pathogenesis of non-infectious pneumonitis, including direct toxicity, formation of immunogenic haptens and increased levels of CD4+ lymphocytes in BAL. It has been suggested that non-infectious pneumonitis typically occurs at the 2–6 month mark but A/Prof Isbel has observed it in patients at the 5+ year mark. The key is to have a high index of suspicion for non-infectious pneumonitis among all clinicians treating a patient, and patients and their GP should be warned about the risks of this event at commencement of drug therapy. Patients may be asymptomatic with radiographic evidence only; ground glass opacities and focal consolidation can be seen on HRCT. Management of non-infectious pneumonitis includes drug withdrawal, after which improvement is seen within weeks. Complete resolution is usually seen within 6 months. A risk benefit analysis should be performed before restarting.

Hyperlipidaemia is very common but mild in everolimus recipients. mTOR inhibitors reduce the catabolism of circulating lipoproteins, thereby reducing their uptake by adipocytes, and increase triglyceride synthesis. Patients should be given advice regarding lifestyle and have lipid levels monitored annually.

Proteinuria causes a major restriction on the use of everolimus in transplant patients. Potential mechanisms that may cause podocyte injury and potentially secondary focal segmental glomerulosclerosis include decreased VEGF and alteration of the podocyte slit diaphragm. Proteinuria should be monitored long-term and its management is unknown. ACE inhibitors are associated with an increased risk of angioedema when combined with mTOR inhibitors. If proteinuria increases, dose reduction or withdrawal should be considered.

Haematological adverse effects include microcytic anaemia, thrombocytopenia and leukopenia. These effects are generally mild, dose dependent and reversible on drug withdrawal. These effects are thought to occur because mTOR inhibitors disrupt iron homeostasis and decrease erythroid progenitor cell differentiation.

In wound healing, mTOR inhibitors block growth signals directing the proliferation of endothelial cells and fibroblasts, and inhibit VEGF and angiogenesis. This can result in hernia and wound dehiscence. Studies from transplant literature suggest this may be dose related but data are confounded by use of other immunosuppressants, diabetes and age. mTOR inhibitors should be withdrawn prior to major surgery where consequences of poor healing are greater than the risk of temporarily withholding drug therapy – e.g. bowel and heart surgery.

Other adverse effects associated with mTOR inhibitors include headache, phosphate wasting, fatigue and lymphoedema. A/Prof Isbel's patients in particular have had a major problem with headache, which has been dose-limiting. Phosphate wasting can be severe in patients on mTOR inhibitors; mTOR is a nutrient sensor and regulates both intestinal and tubular phosphate uptake.

Before treatment starts, A/Prof Isbel's approach is to educate the patient, family and GP about potential adverse effects. She focusses on: stomatitis – they will get it; a management plan for infections and potential pneumonitis; a management plan for surgery; and advice on contraception. TSC patients need plenty of reassurance while transitioning on to treatment. She finds her patients want to be on drug and don't want to come off it.

Summary

- mTOR adverse effects are common but usually mild
- Serious adverse events can occur
- True long term data is lacking
- Therapeutic drug monitoring and surveillance for adverse events should be routine and is the responsibility of the prescriber

Conclusion

Awareness of the full spectrum of TSC disease manifestations and working in multidisciplinary teams and sharing experience will improve outcomes for patients with TSC. One of the first things clinicians can do for their patients with TSC is to get a group of interested parties together from across Australia and decide how to move forward. Extremely good TSC resources are available in Australia for patients, families, GPs and specialists. Having a team of specialists together in one place as exists at Sydney Children's Hospital Randwick and Princess Alexandra Hospital is certainly a promising development and is ideal for patients. How can a surveillance plan for TSC be implemented? Clinicians all have different systems within their own hospitals and within states. It was pointed out that France has a very good TSC surveillance programme that could be adapted for use in Australia. TSC patients need the care of several healthcare providers so the ability to visit multiple specialists on one day instead of having different appointments on different days with various specialists would be a major step forward for patients and their families.

Question & Answer session

I have three dominant families with no mutations in TSC1 or TSC2. Is there a third gene?

(David Mowat [DM]) Most patients with no mutation identified will be mosaic. They will probably have mutations in or around the known genes. If tissue is taken out, it should be analysed for mutations. There is no evidence yet for a third genetic mutation.

How many neurological complications in TSC are related to mutations?

(John Lawson [JL]) Tumours detected in TSC patients show LOH, so there is a presumed 'double hit' in those tumours, i.e. SEGAs, AMLs and skin lesions. In tubers, there is usually no LOH.

What are the neurological consequences of a transcallosal approach to a SEGA?

(JL) Such surgery is just dividing the callosal fibers, so it really has no impact at all. It's only if local structures are affected through venous infarction of basal ganglia or direct injury of the fornices; this may result in amnesia.

Dr Wood, what percentage of urologists know what you know?

(Simon Wood [SW]) Urologists know about treating non-TSC associated sporadic AMLs because they are much more common and come to attention in clinical practice. The rule of thumb is if they're 4cm or bigger you need to treat them. So most urologists would simply transfer this thinking to the TSC patient. But we are hoping to change that.

With sporadic AML, is it TSC? How do you make sure it is just sporadic?

(SW) There are some that are very difficult to diagnose – when Nikky Isbel and I first started seeing these patients, we struggled to establish the diagnosis in some patients and had to enlist the help of geneticists. But if you have an older person with a solitary AML and no other manifestations of the disease it's usually not TSC associated.

I have a 35-year-old with enormous kidneys and moderate mental retardation. Nikky, you've seen 7 patients — how many have you not treated and how do you decide whether to not treat them?

(Nikky Isbel [NI]) My patients see Simon Wood first, so I get a 'biased' referral. Simon, you're probably best to answer how many you see and don't treat.

(SW) My referral pattern is also quite biased in that I get sent the patients no-one else wants anymore because they've taken a kidney out but there are still AMLs – what are they going to do now? I see people who are in trouble and they often need drug treatment. Has the patient had any bleeds or pain from their lesions? (None). Have they got a heavy burden of disease? (Huge). Are the lesions very large? (Yes). Have you got serial imaging? (Only a year, and they're the same). Normal renal function? (Yes). Has there been an assessment for vascular malformation? (No). If he has stable lesions and is otherwise healthy, I would monitor lesions. The trigger for treatment may be pain, haematuria or lesion growth. The risk of a sudden catastrophic bleed is small but as he lives remotely, this may be a reason to favour treatment. The wrong thing is to take out kidney. As we've heard today, it is a complex risk:benefit assessment.

Should he and his brother have gene testing? His brother appears to be completely normal.

(DM) TSC is a clinical diagnosis primarily, not genetic. We would look at the parents' skin, do cerebral imaging and renal ultrasound. If siblings are worried, I recommend they talk to a specialist and get a clinical diagnosis. The chance of someone having no skin signs as an adult and no other features and having TSC is extremely remote. 25% of patients don't have mutations identified anyway and we still have to resort to the clinical scenario.

(Sean Kennedy [SK]) My inclination would be to treat your patient. We don't know the incidence of chronic kidney disease leading to ESRF. We don't know the event rate for bleeding and that is a risk for him. I have seen some older patients with kidneys like his whose GFR is dropping and they've got proteinuria. The biggest risk for reducing GFR is intervention. But I think if a patient's kidney is gradually being replaced by AMLs their GFR will drop eventually.

(NI) But we have no knowledge that an mTOR inhibitor alters that natural history at all. When we put patients with these enormous lesions on drug therapy, what is the endpoint? We make the lesions decrease, say from 12cm to 9cm, but what is the endpoint – do we then increase drug again? I don't think there is enough evidence to push for the long term preservation of renal function as an endpoint. It would be great to find out if everolimus does preserve long term renal function. If he gets pneumonitis when out in country and is not diagnosed.... there are risks.

(SK) I would give him a 6–12 month treatment trial and see how he feels.

My patient is in her late 20's, has ESRF, bilateral nephrectomy, failed transplant, mild cognitive impairment, and is on home dialysis in the country by herself. She has a boyfriend with cerebral palsy. This raises a whole host of questions about surveillance, genetic issues and contraception. I would be interested in the panel's comments.

(DM) If they want to have a family, they should be able to explore that. There are ways of having children and avoiding TSC in their children. TSC and dialysis are not necessarily reasons to not have a family; it's just a bit complex.

(Deborah Yates [DY]) Having a family is very much an individual decision. I think it would be worthwhile doing lung function tests and a CT because if she has LAM this would alter the balance towards not bearing a child. Nonetheless, avenues need to be explored for her if that's what she wants. Contraception is very dependent on whether she's got LAM or not. CT is difficult to access in some country areas, but a lung function test would be a beginning at least.

If there is no LAM, is the oestrogen-containing oral contraceptive ok for females?

(DY) Yes. A lot of LAM cases are actually oestrogen and progesterone-receptor positive. Progesterone is meant to be protective in this regard. It's lack of an oestrogen-containing contraceptive which is important to have. I think there needs to be a better evidence base on the contraceptive pill in LAM. We have a number of patients who have started themselves on the pill and haven't actually had any decline in lung function. The levels of oestrogen in contraceptive pills are actually extremely low compared to levels reached in pregnancy. So this is really an open area.

(SK) There is a possibility that AMLs may also be oestrogen responsive.

(DM) Two parents with potential genetic disorders is par for the course in my clinic. We always hope for people to make their own decisions regarding starting a family.

Some of these patients are going through pre-implantation genetic diagnosis; what happens to their LAMs when given ovarian stimulating agents?

(DY) We haven't had any patients in this situation yet. One patient is considering pre-implantation genetic diagnosis and the recommendation from her obstetrician is to allow a normal cycle. Provided women are young enough they can apparently harvest enough eggs during a normal cycle. It's when they get to their late thirties that ovarian stimulating agents are often required. We would recommend patients having such procedures earlier rather than later to avoid ovarian stimulating agents.

Can I ask about the registry – is everyone in it and how extensive is the information collected in it?

(DM) There is no registry in Australia. There is European/Australian registry called TOSCA which has 2000 patients. There is a natural history TSC database in the US through the TS Alliance. My idea of a registry is that everyone with TSC is best to be on the register providing they give consent. There is value in having large numbers of patients. To manage one particular cohort we need a recall system. There is a good argument to have a registry for TSC in Australia, which is all about patient sign on and making themselves available for potential future therapies. That's what people want – they want to know they can access therapies.

(DY) There is an international LAM registry run primarily from the LAM Foundation in the US. We report our data to them. The LAM Foundation is a patient-based registry and information is sent from this to designated LAM clinics. We did originally collect data from Australian LAM patients, but this was superseded by an orphan lung disease registry. The difficulty on the whole is that the LAM registry is a patient notification database and diagnosis may not be entirely accurate. The registry has great advantages in terms of allowing patients access to trials. The local LAM Foundation does have a list of patients but it is very inaccurate. The whole thing needs to be coordinated better and then we will be in a much better position, not only from the point of view of allowing access to clinical trials but also in terms of support. Registries can be useful and while easy to set up are difficult to continue running due to lack of funding.

What is the preparation for transplant with AML patients? Do you take AMLs out prior to transplant? If not, is there any monitoring and is there any risk of them transforming to malignancy post-transplant?

(NI) We stopped taking AMLs out prior to transplant a few years ago. We do an MRI pre-transplant and generally leave them alone. It does bring up the interesting concept of whether patients should go on an mTOR inhibitor post-transplant. If they're on immunosuppressants, mTOR inhibitors can be used safely. What is being prevented in these patients I'm not sure. The most interesting question is in lung transplant patients and whether or not going onto an mTOR inhibitor once their wounds have healed is recommended.

(DY) This issue in lung transplant is controversial. There is a general consensus that mTOR inhibition should be ceased before transplant. There has been quite a bit of concern over the higher rate of fall in FEV_1 in patients on transplant waiting lists who have their mTOR inhibitor stopped. So the current consensus, which is very much under discussion at the moment, is should they be switched from sirolimus to everolimus because it has a shorter half-life? Then the drug could be stopped a few days before transplant rather than weeks or months before transplant. Anecdotally, everolimus would be the agent of choice.

(DM) No-one needs a transplant providing TSC is managed properly. No doubt better therapies are coming.

(SK) Certainly in TSC patients who develop ESRF early, we have no idea whether everolimus would make a difference to them.

(SW) If someone with TSC does have a renal transplant, it means they've had a lot of trouble from their kidneys. I don't want to give the opinion that we'd never treat. If someone has had multiple bleeds/embolisation, and then transplant, in that scenario a laparoscopic nephrectomy would be a very good treatment. We don't tend to worry about malignant transformation in renal AMLs; it's essentially unheard of. Malignancy can be discounted from any fat-containing lesion.

Is there any evidence of tailoring therapy for TSC in different organs? How did the current dose get arrived at?

(NI) The current dose came from oncology literature, based on enzyme inhibition. Dosing and dose exposure is not clear in registry studies. Patients who are also on anti-epilepsy drugs have altered drug exposure. The answer is we don't know.

(JL) In SEGA trials a fixed dose was used. But out of trials we start at a low dose, half or even less and see how it goes. We get an MRI scan in 3 months. The therapeutic target in trials is 5–15 ng/mL but some patients have done well on much lower doses. In SEGAs, response is easy to measure, where a positive response is lack of growth. It is harder to measure response in epilepsy, autism etc.

(DY) From the point of view of the lungs, we are paranoid about pneumonitis and I think it occurs much more commonly than people think. There is lack of baseline data in the majority of trials. There was a study conducted about 8 years ago in our hospital where the transplant service tried everolimus for interstitial pulmonary fibrosis. They used 5–10mg and were aiming for a trough of 10ng/mL. There was a very high incidence of toxicity in the everolimus group including lung toxicity. Because of that study we now treat at much lower level. We run our patients at a 2–5 ng/mL trough level, based on symptoms. It is difficult to know whether they would do better at higher doses and we have had cases of lung toxicity even at very low doses, so they probably have pre-existing abnormal lungs. So for the lungs, I don't think you need a high dose and I think there are risks with high doses.

(DM) The other aspect to your question about individual variation is that I think there is tissue specific variation in individuals which we currently have no capacity to measure. We will gradually get a handle on personalising medicine to the individual organ as time goes on.

(NI) What are you treating the patient for? What is the endpoint? We know that this drug prevents growth. If you have a younger person with evidence of growing lesions, then prevention is very important. In those cases you would be prepared to push the dose harder. The other question is, what do we all do after a patient has achieved response? What is the maintenance approach?

(JL) Regarding stomatitis, we have treated about 25 children with mTOR inhibitors and haven't had to reduce the dose in any for stomatitis. We've had to temporarily stop the drug, but haven't had to permanently withdraw it.

(SK) We know that lesions are organ specific. Cardiac rhabdomyomas are present at birth then disappear, SEGAs grown in childhood then slow down, and AMLs don't grow much until later on. In our experience, when we started treating SEGAs off-label before the trials, we started at the transplant dose (a low dose) and saw an effect. In my smaller experience in AMLs we don't see the same effect at a lower dose.

(DM) The same is true with the topical agent. To see an effect in an adult with extensive angiofibromas, a high dose is needed. But once a good response is obtained, one can drop down to a low dose.

One of the problems we have in South Australia with managing epilepsy is access to a ketogenic diet clinic. What is the efficacy of ketogenic diet versus mTOR inhibitor in patients not managed successfully with surgery?

(JL) If we are talking about our typical preschool child with intractable epilepsy, I would always go to a ketogenic diet before an mTOR inhibitor. And in fact I would often choose diet before surgery too, partly because I find it very difficult to isolate a tuber in this age group. Ketogenic diet is a temporary measure – we use it for about 2 years. It works on the mTOR pathway and can achieve dramatic results. Parents like a natural approach.

So if you've made the decision to then give an mTOR inhibitor would you then take a patient off a ketogenic diet?

(JL) No. There is anecdotal evidence that the two do work synergistically.

Have you seen any pancreatitis in patients on ketogenic diet?

(JL) No. At Sydney Children's Hospital Randwick we do a minimum of one ketogenic diet per month for intractable epilepsy. About one of those patients per year has TSC and about once a year we would see a patient with extraordinarily high triglyceride levels, but we haven't seen pancreatitis yet.

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