What's new in TSC???

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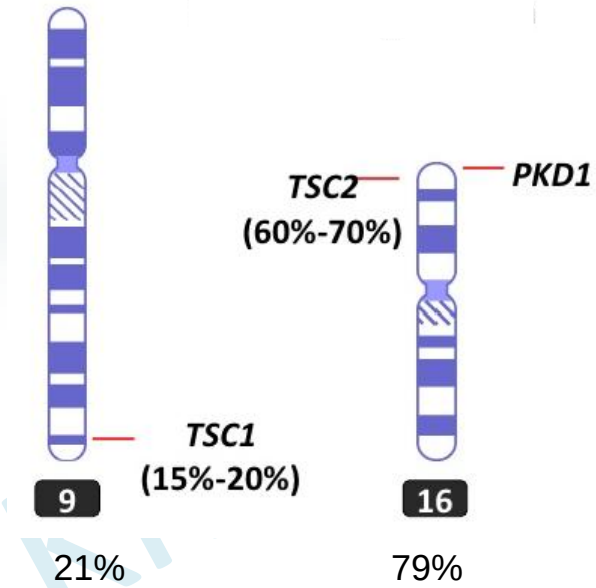
BASICS TSC

- TSC is a rare autosomal dominant genetic disorder (1/8000) characterised by development of benign tumours and lesions in various organs
- TSC is caused by mutations in the *TSC1* or *TSC2* tumour suppressor genes, which code for hamartin and tuberin, respectively
- Hamartin and tuberin form a complex that indirectly inhibits the activity of the mechanistic target of rapamycin (mTOR)

Basic genetics of TSC



TSC1: 23 exons, 130 kDa protein, 1,164 aa
TSC2: 41 exons, 200 kDa protein, 1,807 aa

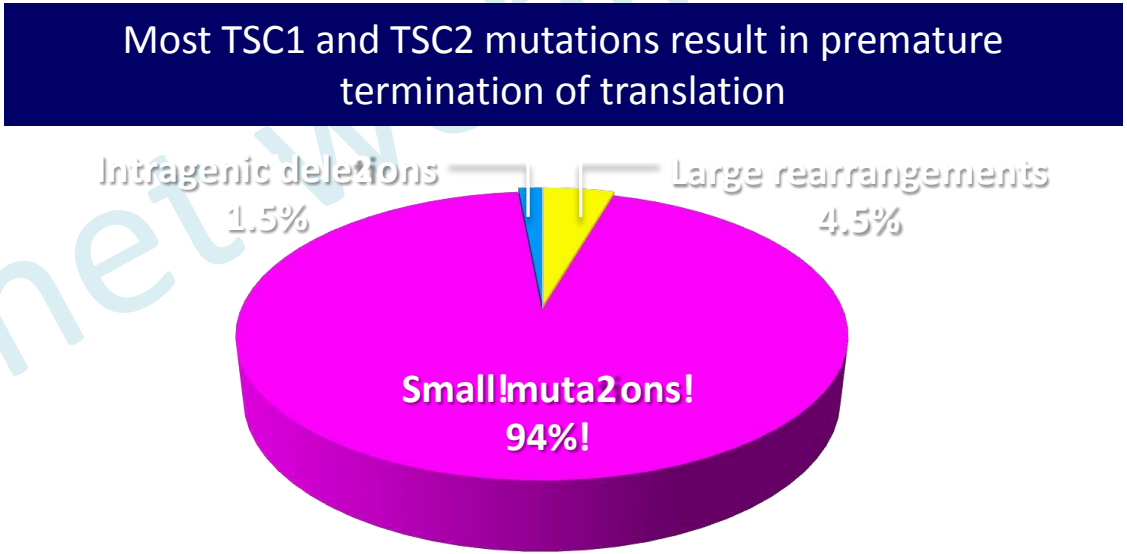
- Autosomal-dominant ~ 100% penetrance
- More than 2,000 non-synonymous mutations have been identified in *TSC1/TSC2* genes
  - 50–60% of all mutations are single-base substitution mutations (C < T)
  - large rearrangements: 6% *TSC2*, 0.5% *TSC1*
- 10–15% NMI
- 2/3 are *de novo* mutations
- Only genotype–phenotype correlation
  - CGS
  - some missense mutations in *TSC2*-mild

aa, amino acids; CGS, contiguous gene syndrome; NMI, no mutation identified; TSC, tuberous sclerosis complex.

Kozlowski P, et al. Hum Genet. 2007;121:389-400.
Kwiatkowski DJ, et al., editors. Tuberous sclerosis complex: genes, clinical features, and therapeutics. Weinheim: Wiley-VCH Verlag; 2010.
Qin W, et al. Hum Genet. 2010;127:573-82.

MUTATIONS IN TSC

- Identified in 70-90%. Otherwise NMI. Cause? Non coding regions/ Mosaicism mostly



NGS in TSC

ADVANTAGES:

- Lower cost
- Quicker
- Mosaicism can be detected

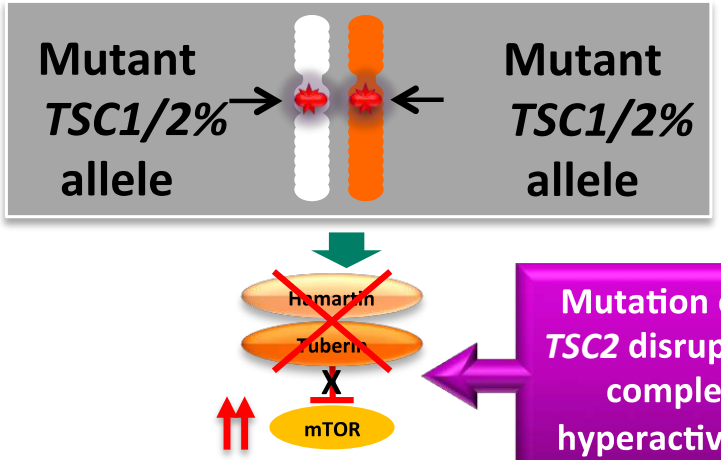
DRAWBACKS:

- Large in/del
- High number of variants per individual (prioritisation). No functional test available. Difficulty classifying variants. Patient derived cells for RNA-based studies would greatly facilitate these studies
- Mosaics may be missed in regions with low coverage
- Wait for large number of individuals to be tested in a single run to optimize cost (avoided with panels)

MOLECULAR PATHOLOGY OF TSC

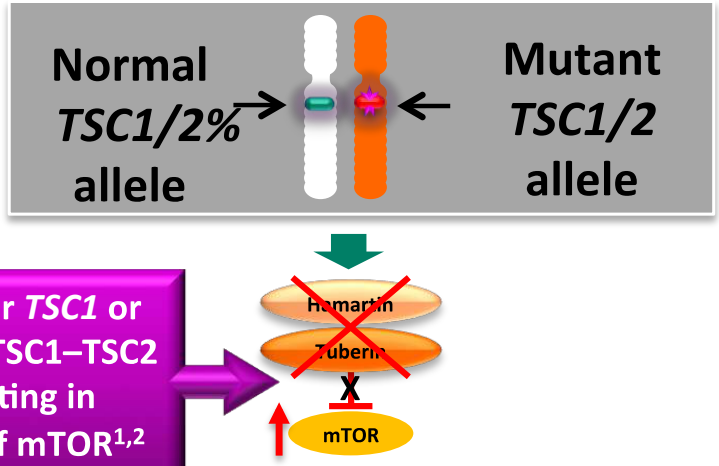
Loss of Heterozygosity (LOH)[2]

Inactivation of second allele of *TSC1* or *TSC2*



Haploinsufficiency[2]

Inactivation of a single allele of either *TSC1* or *TSC2*



Mutation of either *TSC1* or *TSC2* disrupts the TSC1–TSC2 complex, resulting in hyperactivation of mTOR[1,2]

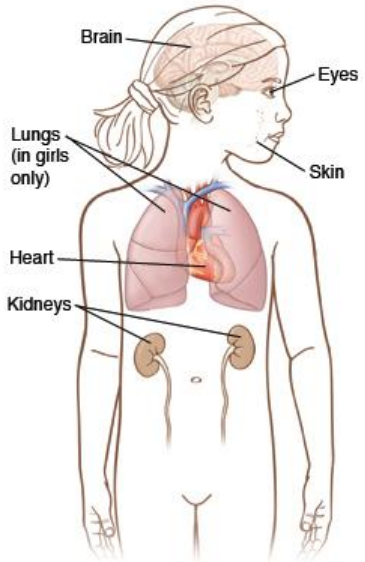
TSC is SYSTEMIC disease

Major features

1. Hypomelanotic macules (3, at least 5-mm diameter)
2. Angiofibromas (3) or fibrous cephalic plaque
3. Ungual fibromas (2)
4. Shagreen patch
5. Multiple retinal hamartomas
6. Cortical dysplasias*
7. Subependymal nodules
8. Subependymal giant cell astrocytoma
9. Cardiac rhabdomyoma
10. Lymphangioleiomyomatosis (LAM)
11. Angiomyolipomas (2)

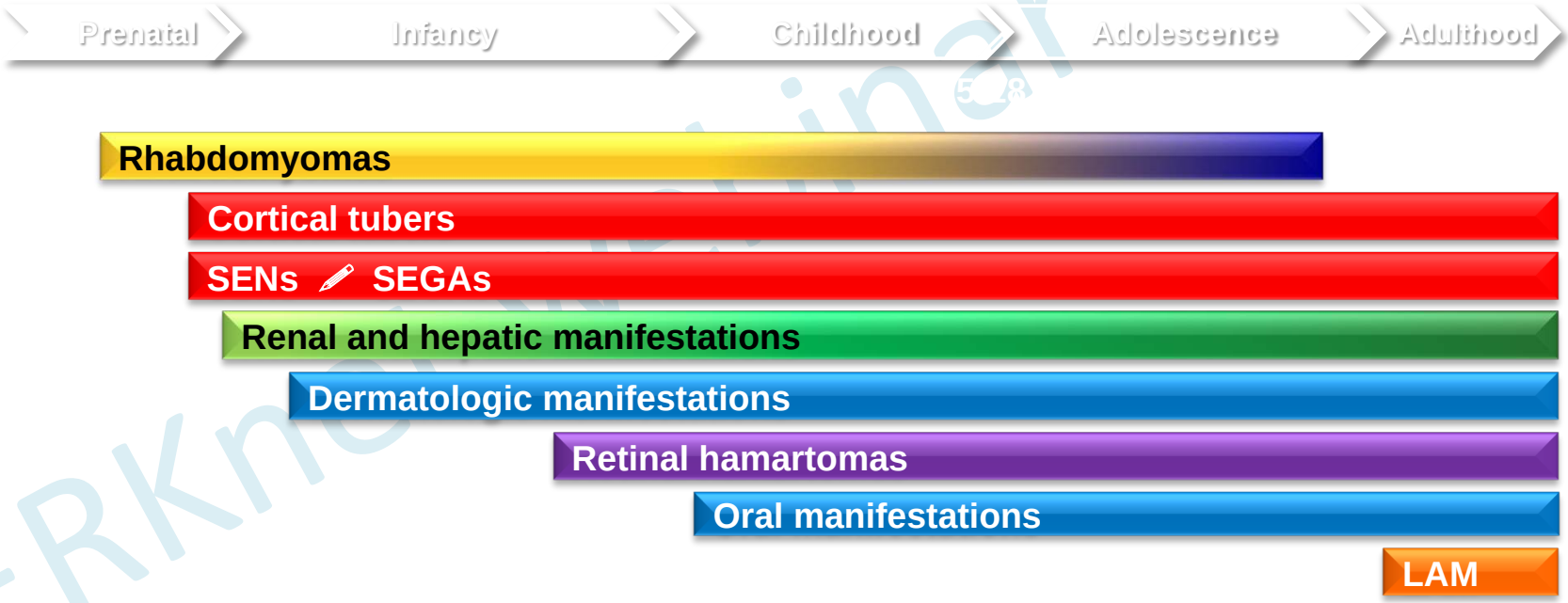
Minor features

1. “Confetti” skin lesions
2. Dental enamel pits (>3)
3. Intraoral fibromas (2)
4. Retinal achromic patch
5. Multiple renal cysts
6. Nonrenal hamartoma

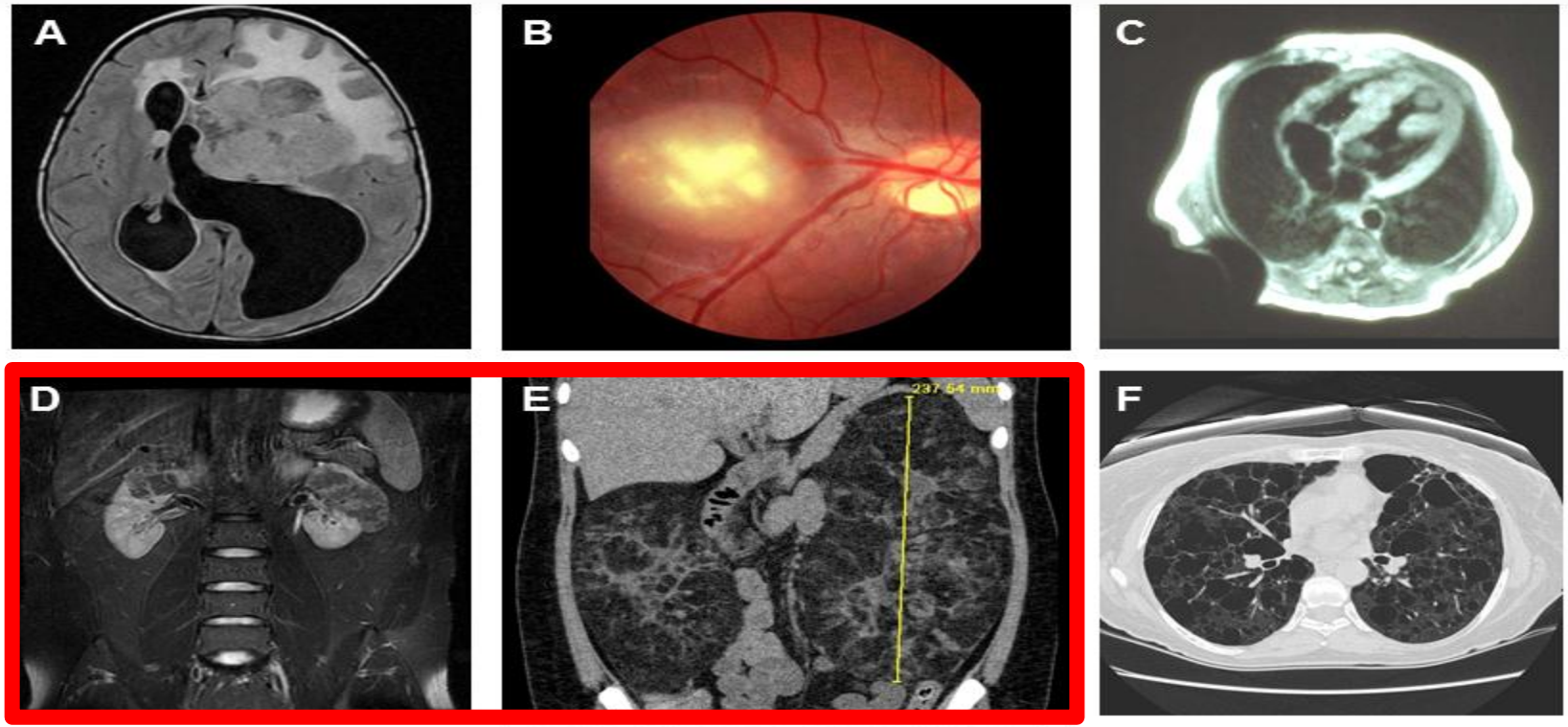


Northrup et al, Pediatric Neurology 49 (2013) 243e254

CLINICAL PRESENTATIONS OVER TIME



CLINICAL FEATURES OF TSC



Images courtesy of John J. Bissler, MD.

Renal involvement in TSC

CYSTS

- No clinical repercussion except for the contiguous gene syndrome: CGS *TSC2/PKD1*
- More frequent with *TSC2* mutations

ANGIOMIOLIPOMAS (AML)

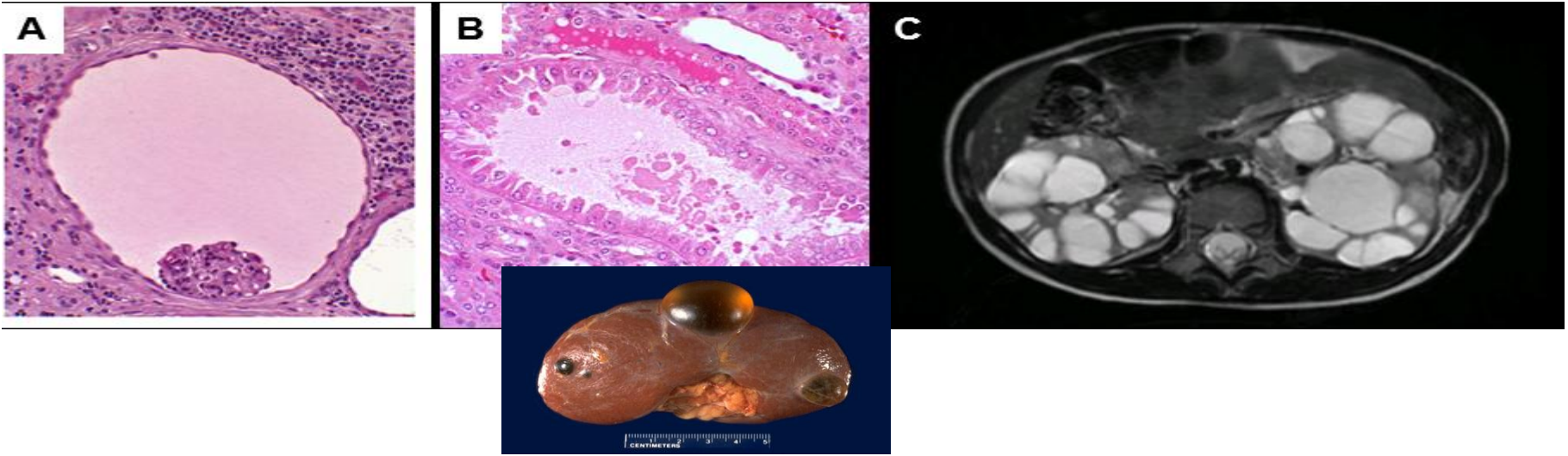
- More frequent and severe in *TSC2*
- Clinical repercussion

RENAL CANCER

-Infrequent

-Difficult to diagnose

CYSTS IN TSC



B

A- Glomerulocystic disease. Infrequent but not easy to dignose

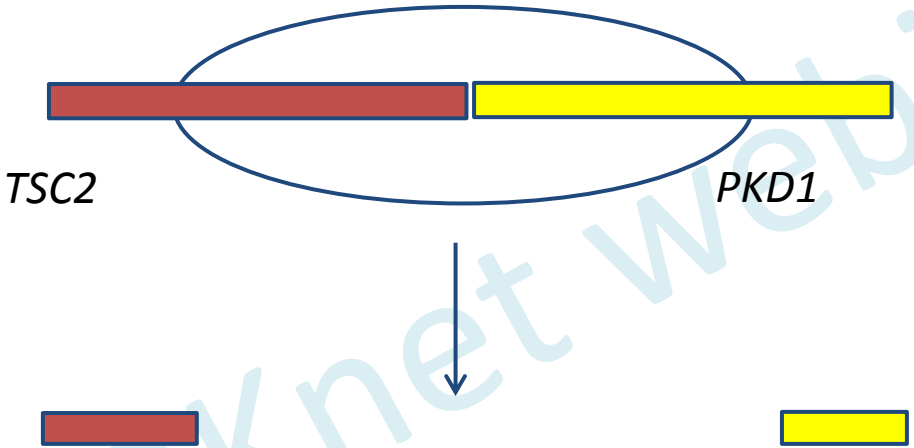
B- Simple cysts: micro/macro: 30-50% of patients

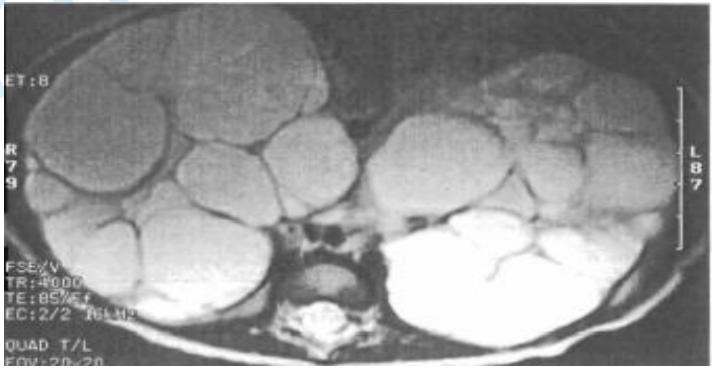
C- CGS *TSC2/PKD1*

Modified fom Bissler et al 2010 Pediatr Npehrol

Renal Cysts in Patients With TSC

TSC2/ PKD1 contiguous gene deletion Sd





Torra R et al 1998

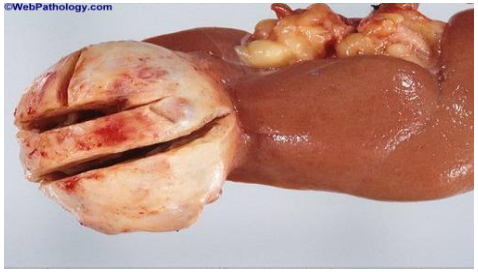
2– 5 % of TSC patients**:**

Severe, very early onset **PKD**

Significant **CKD in teenage years**

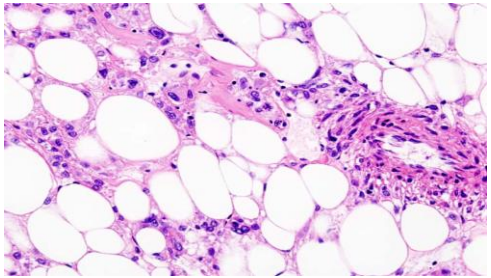
AMLs in Patients With TSC

AML represent 1-2% of all renal tumors. 0.13% of population have AML. 20% of patients with AML have TSC.



AMLs develop in up to 80% of patients with TSC

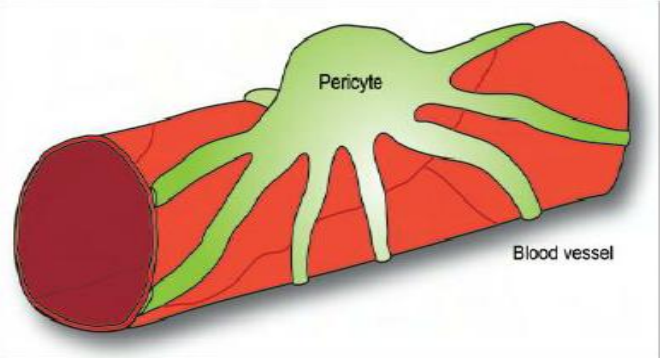
- Multiple and bilateral renal tumors
- 8.6 years = median age of initial AML detection
- Benign hamartomas rich in fat, blood vessels, and smooth muscle
- May occur in other organs



*Curatolo P et al. Lancet. 2008;372:657-668.*5. Eijkemans Am J Kidney Dis. 2015; 66: 638-45.

Pericyte origin of TSC-associated AML

- Pericytes are mesenchymal perivascular cells attached to the abluminal surface of capillaries.
- Specific functions in regulating microvascular stability, development, and function
- AML cells, like pericytes, histochemically express α-SMA and pericytes also can accumulate lipid, as is seen in AML

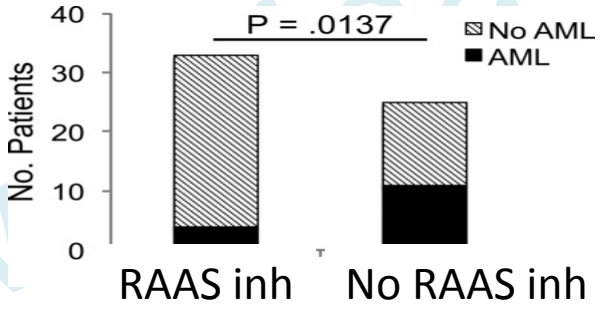


Siroky BJ et al Am J Physiol Renal Physiol. 2014

Pericyte origin of TSC-associated AML

Decreased renal AML development in:

– Patients with CGS *TSC2- PKD1* treated from an early age with ACEI or ARBs due to HBP



TSC-associated AML

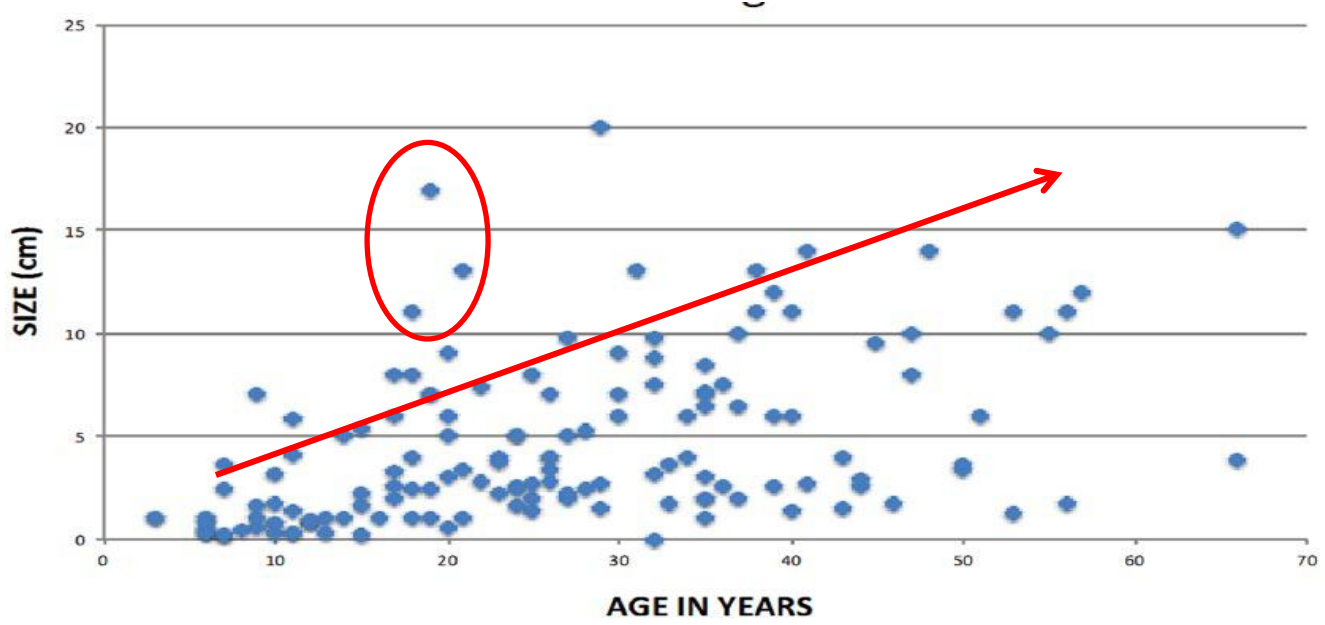
- express:
  - ANG II type 1 receptors
  - platelet-derived growth factor receptor-β
  - desmin
  - α-smooth muscle actin
  - VEGF receptor
- but do not express:
  - adipocyte marker S100
  - endothelial marker CD31

Serum from TSC AML patients has increased:

- VEGF-A
- VEGF-D
- soluble VEGF receptor 2 collagen type IV

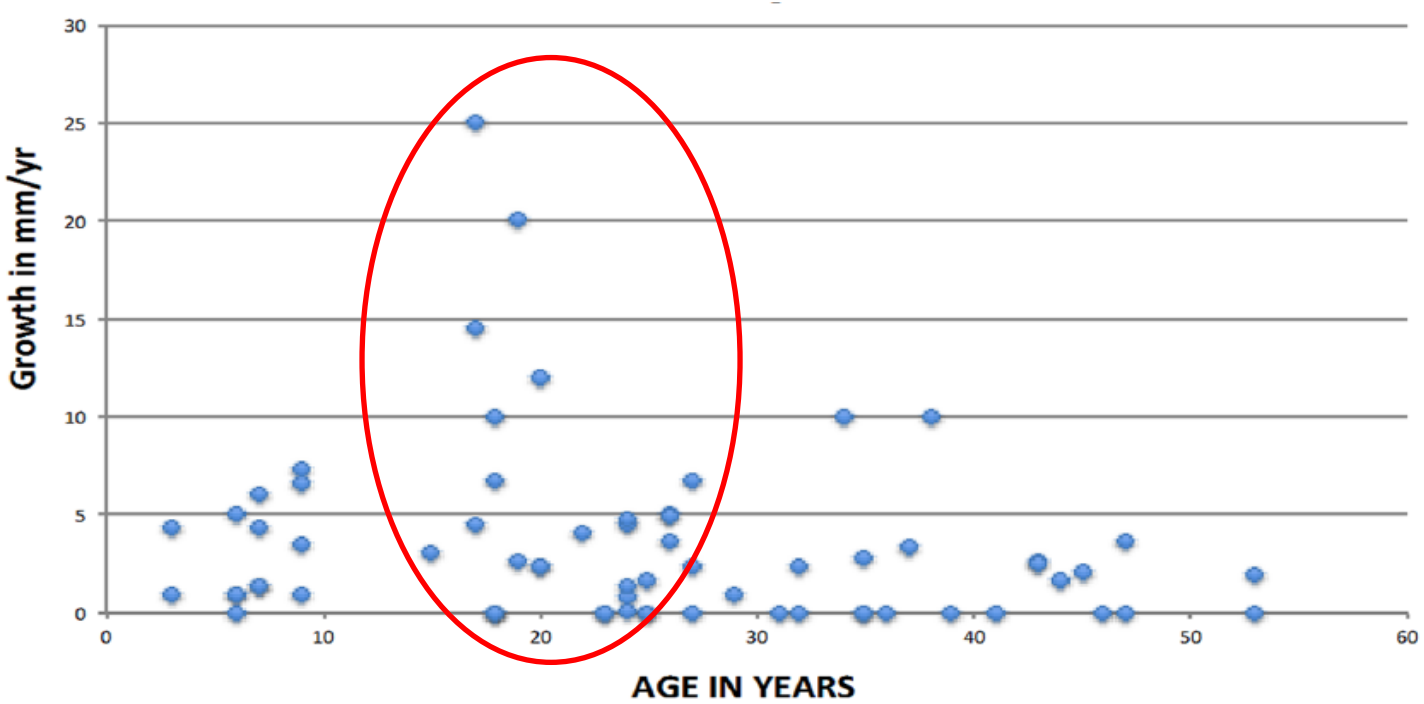
Siroky BJ et al Am J Physiol Renal Physiol. 2014

SIZE OF AML vs AGE



Cox et al 2012

RATE OF AML GROWTH vs AGE



Cox et al 2012

Clinical presentation of AML

- Often discovered as an incidental finding on radiological studies
- Classical triad of presenting signs
  1. **flank pain**
  2. **palpable mass**
  3. **haematuria**

Clinical manifestations of AMLs
• Acute abdominal pain
• Chronic abdominal pain
• Acute flank pain
• Chronic flank pain
• Nausea and vomiting
• Fever
• Shock
• Hypertension
• Tenderness
• Palpable abdominal mass
• Palpable flank mass
• Anaemia
• Renal failure
• Microscopic haematuria
• Gross haematuria
• Urinary tract infection
• Haemorrhage

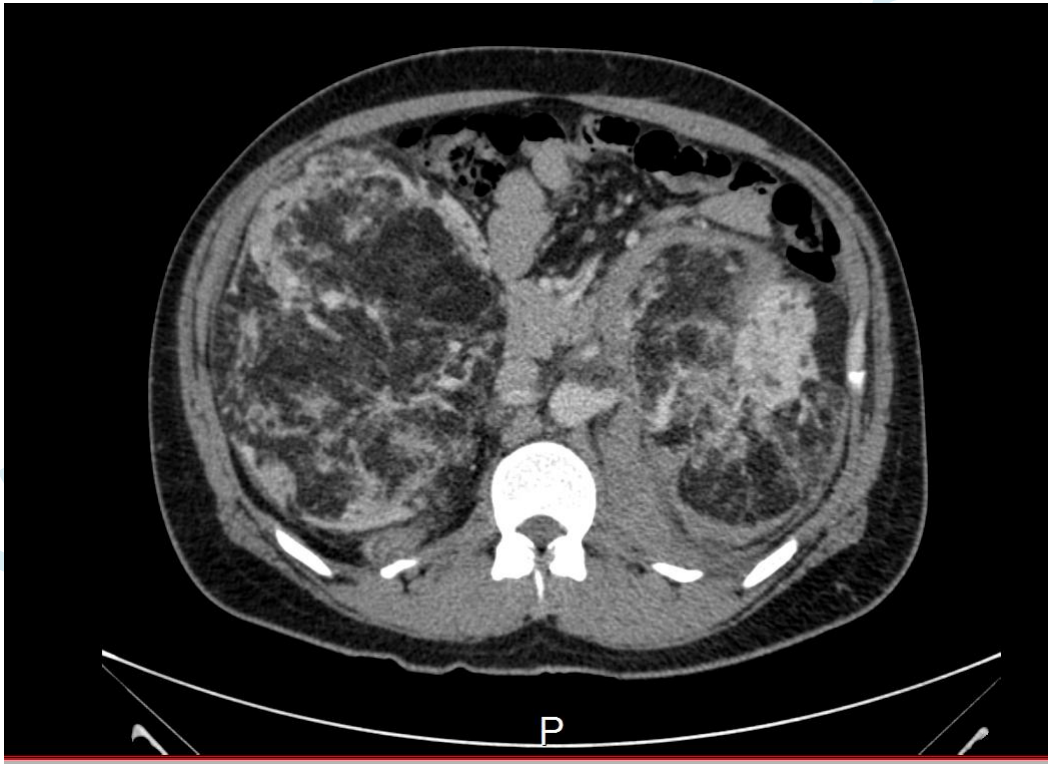
Bissler JJ, Kingswood JC. Kidney Int. 2004; 66(3):924-34

AMLs in Patients With TSC

- **Cumulative risk of hemorrhage** is **18% for women and 8% for men**
  - **Embolisation/nephrectomy in 25% to 50% of patients**
  - Re-embolisation in up to 45% of patients
  - Risk of hemorrhage depends on size of AML (>3cm)
- Encroachment of AMLs on normal tissue may lead to **renal failure**

Song et al CMRO 2017

Encroachment??



Renal Cell Carcinoma in Patients With TSC

- Patients with TSC are at increased risk (?) (**estimated 1%-3%**) of **developing renal cell cancer**
- Histology is quite varied and usually low grade
- Disease develops at an **earlier age**: 30 versus 50 to 60 years, and primarily in women
- Especially fat-poor AML sometimes difficult to distinguish in MRI scan: experienced radiologist ± tumor biopsy

Bissler and Kingswood 2016

ESKD in Patients With TSC

AJKD
Original Investigation

Long-term Follow-up Assessing Renal Angiomyolipoma Treatment Patterns, Morbidity, and Mortality: An Observational Study in Tuberous Sclerosis Complex Patients in the Netherlands

Marinus J.C. Eijkemans, PhD,[1] Willem van der Wal, PhD,[1] Leida J. Reijnders, MSc,[2] Kit C.B. Roes, PhD,[1] Sahar Barjesteh van Waalwijk van Doorn-Khosrovani, PharmD, PhD,[3] Corey Pelletier, PhD,[4] Matthew Magestro, MS,[4] and Bernard Zonnenberg, MD, PhD[2]

Eijkemans Am J Kidney Dis. 2015; 66: 638-45.

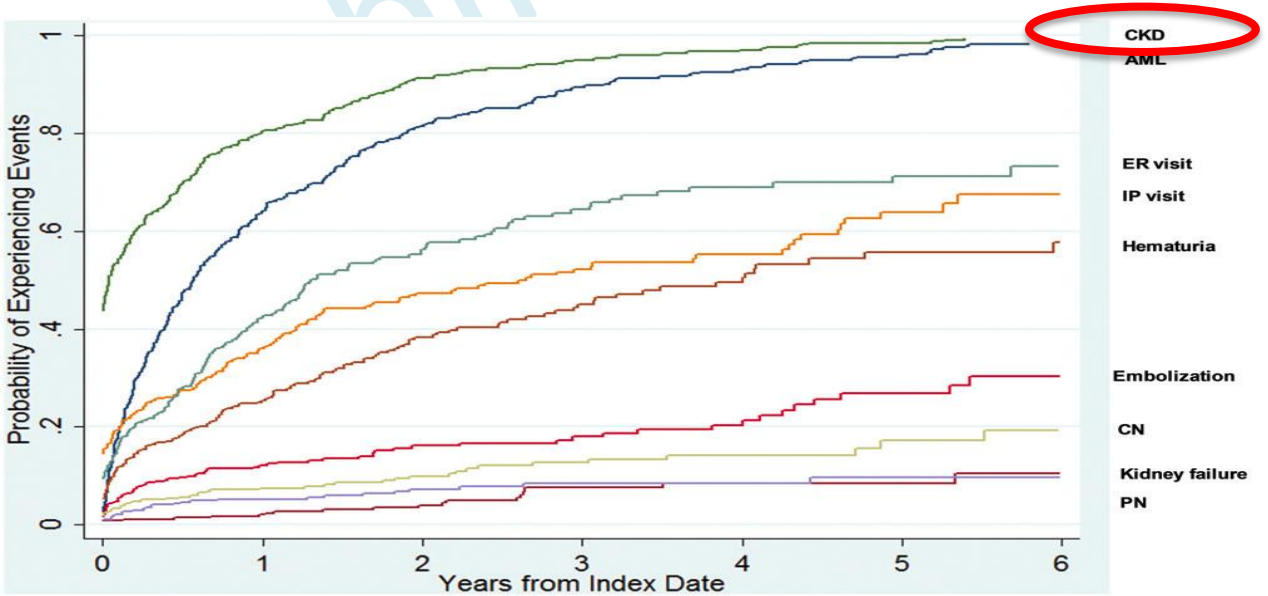
244 TSC patients with AML (1990-2012):

7 dialysis
7 transplantation
4 death with ESRD

ESRD: 18/244 = 7.3%

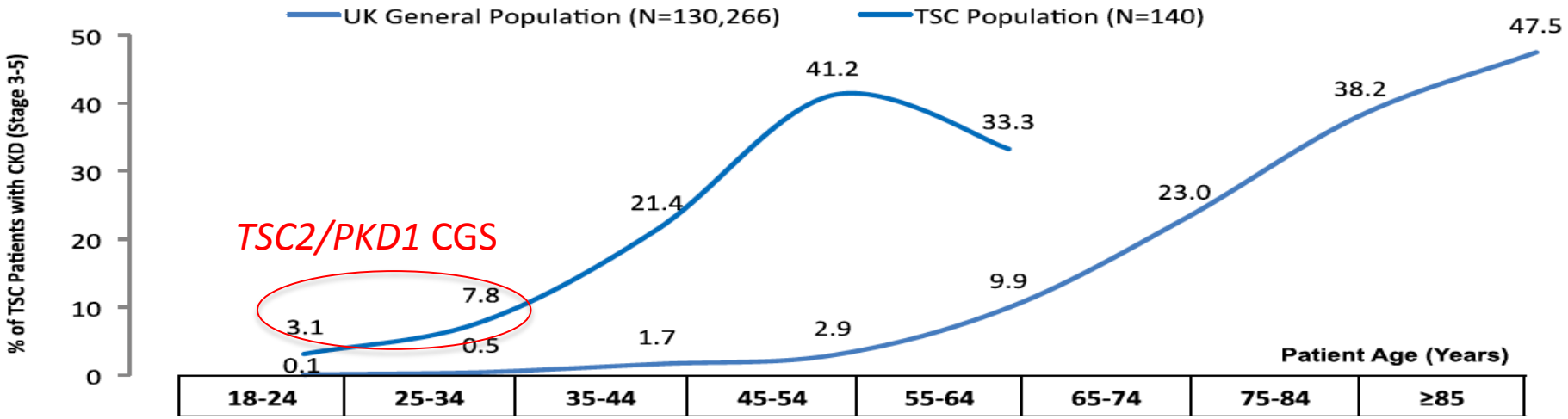
Natural history in TSC-AML

- 605 patients were selected (<18 years N.225; 18 years N.380)
- CKD occurred in 12.4% of patients <18 ys (CGS) and 23.4% of patients >18 ys
- Some functional CKD to occur in almost all patients within 6 years of diagnosis.



Song et al CMRO 2017

TSC-CKD



Prevalence of CKD in the overall TSC population by age compared with the general UK population

Kingswood et al 2014

Whats is the cause of CKD in Patients With TSC

- CGS ***PKD1-TSC2***
- Loss of renal parenchyma due to **embolizations or nephrectomies**
- **Encroachment** of renal parenchyma by AML
- Glomerulocystic kidney disease?

- Somatic **second-hit** mutations occurring during rapid cell division (when the kidney still has growth and repair potential at age <35–40) may cause an accelerated **loss of normal renal tissue** leading to CKD.
- *TSC1* or *TSC2* **haploinsufficiency** may lead to modest **mTORC1 overactivity** and, therefore, glomerular hypertrophy and hyperfiltration
- Either **haploinsufficiency or second** hit in the tubule cells could predispose to **premature apoptosis or maldifferentiation**

Kingswood et al Nephron 2016

AML Treatment

- There have not been any controlled trials of embolization, nor any trials to compare treatment modalities (surgery, embolization & mTOR inhibitor treatment).
- Embolization 32% recurrence
- **Risk for bleeding** should be treated
  - Once a diameter of 3-4 cm is reached, complications may develop in 68–80% of patients

- **Surgery and embolization** should be performed as **emergency treatment** in bleeding episodes

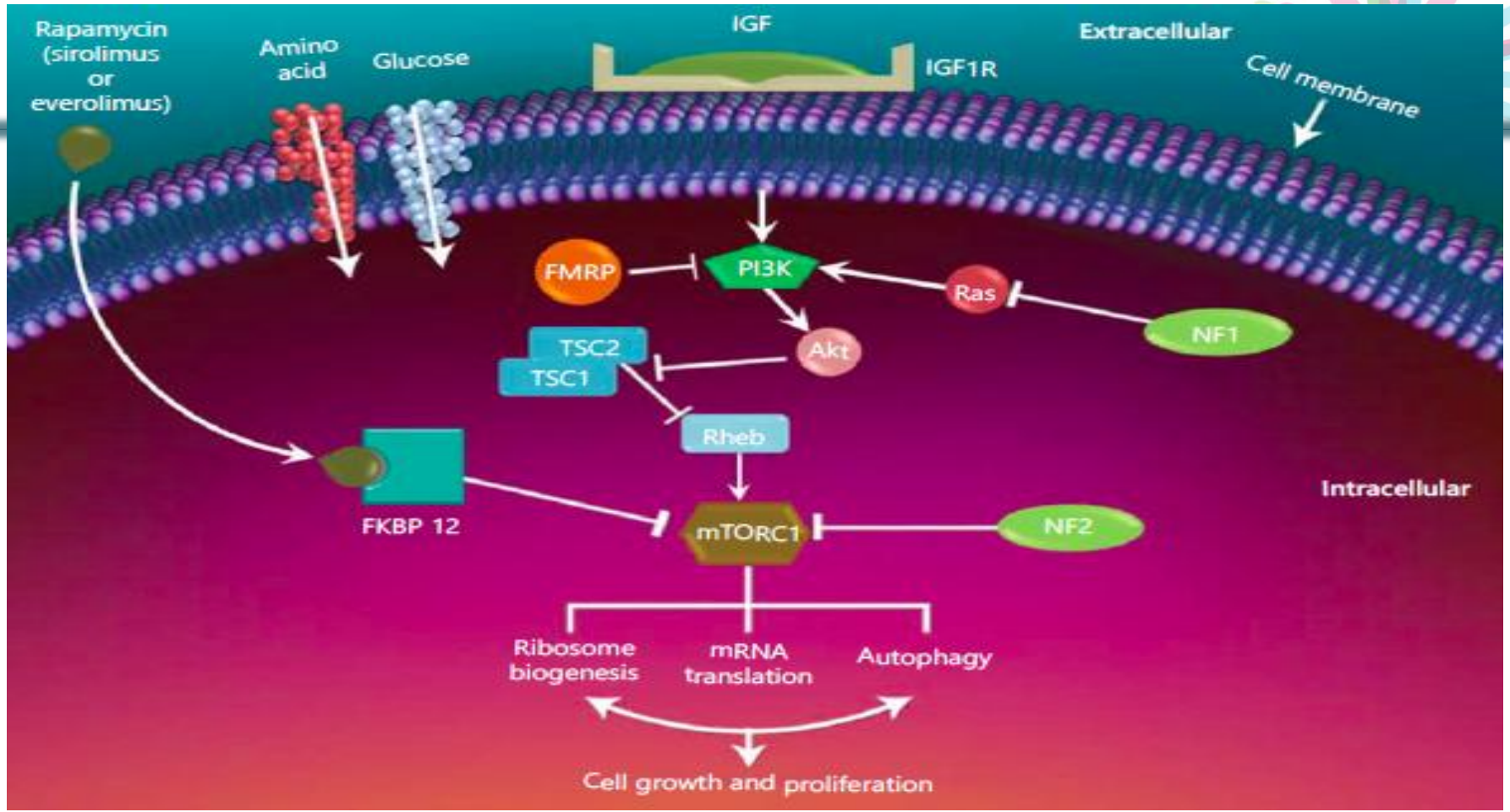
- AML **treatment with mTOR inhibitors** should be initiated in all **elective treatment situations**

Kingswood et al 2016

Treatment decission

- Acute bleeding:
  - Embolization
    - Partial nephrectomy if not available
      - Total nephrectomy if not feasible
- Asymptomatic AML
  - <3cm diameter: follow up
  - >3cm diameter: propose treatment (pros and cons)

Kingswood et al 2016



Kingswood et al 2016

Sirolimus in TSC-AML

	Bissler *et al.* 2008 [1]	Davies *et al.* 2011 [2]	Dabora *et al.* 2011 [3]	Cabrera *et al.* 2011 [4]
	$n = 20$	$n = 16$	$n = 36$	$n = 17$
Patients	6: TSC only	7: TSC only	15: TSC only	all TSC only
	8: TSC + LAM	3: TSC + LAM	21: TSC + LAM	
	6: sporadic LAM	6: sporadic LAM		
Inclusion criterion	≥1 AML ≥1 cm	≥1 AML ≥2 cm	≥1 AML ≥2 cm	≥1 AML >2 cm
Maintenance sirolimus troughlevel (ng/mL)	1–5 in 1	3–6 in 12	3–15	4–8
	10–15 in 19	6–10 in 4		
End point	Total AMLs volume (MRI)	Total AMLs size[a] (MRI)	Total AMLs size[a] (MRI)	Volume of the largest AML (MRI)
Mean decrease in AML volume/size at 12 months	47% in volume	39% in size	30% in size	66% in volume

[a]As defined by the sum of the longest diameters of all target AMLs.

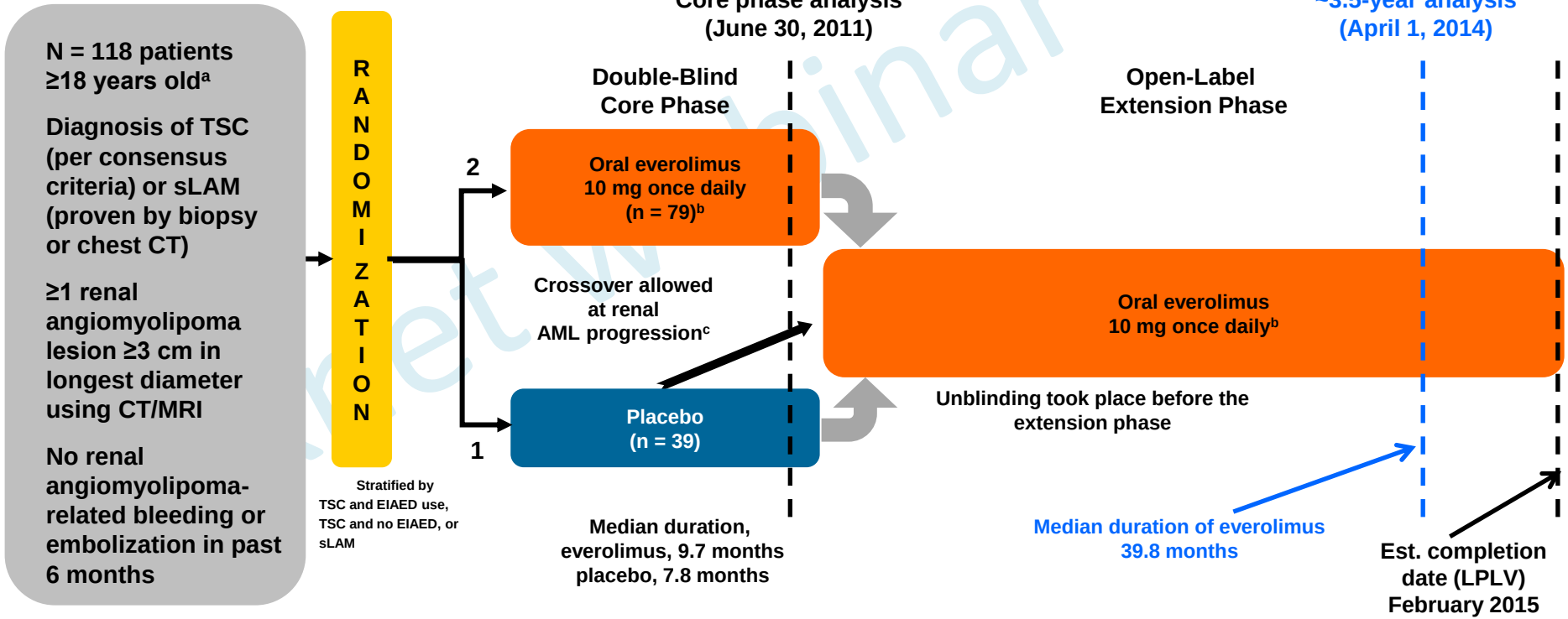
Pirson NDT 2013

EXIST-2

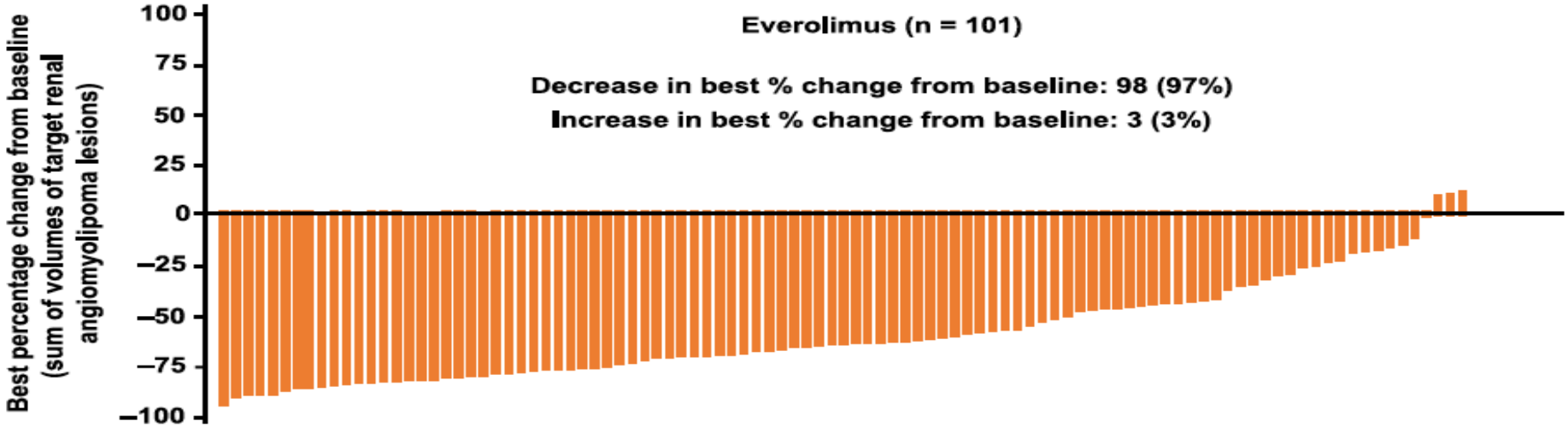
EXIST-2: Phase III, Multicenter, Placebo-Controlled Study in AML EVEROLIMUS

J.C. Kingswood, K. Budde, B. Zonnenberg, M. Frost, E. Belousova, M. Sauter, A. Nonomura, M. Bebin, Y. Pei, T. Sahmoud, G. Shah, D. Gray, J. Bissler

EXIST-2 design



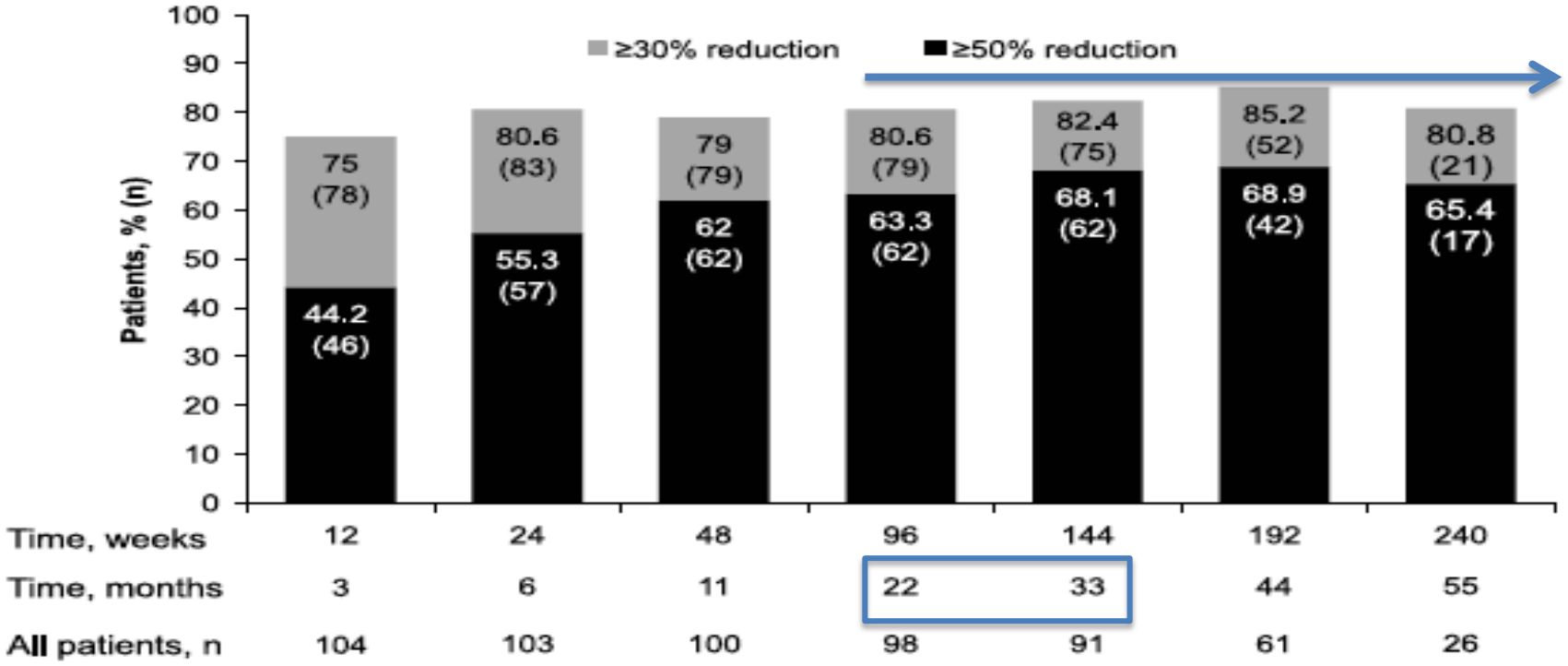
Long term effect of everolimus in AML



MEDIAN: 47 months on everolimus

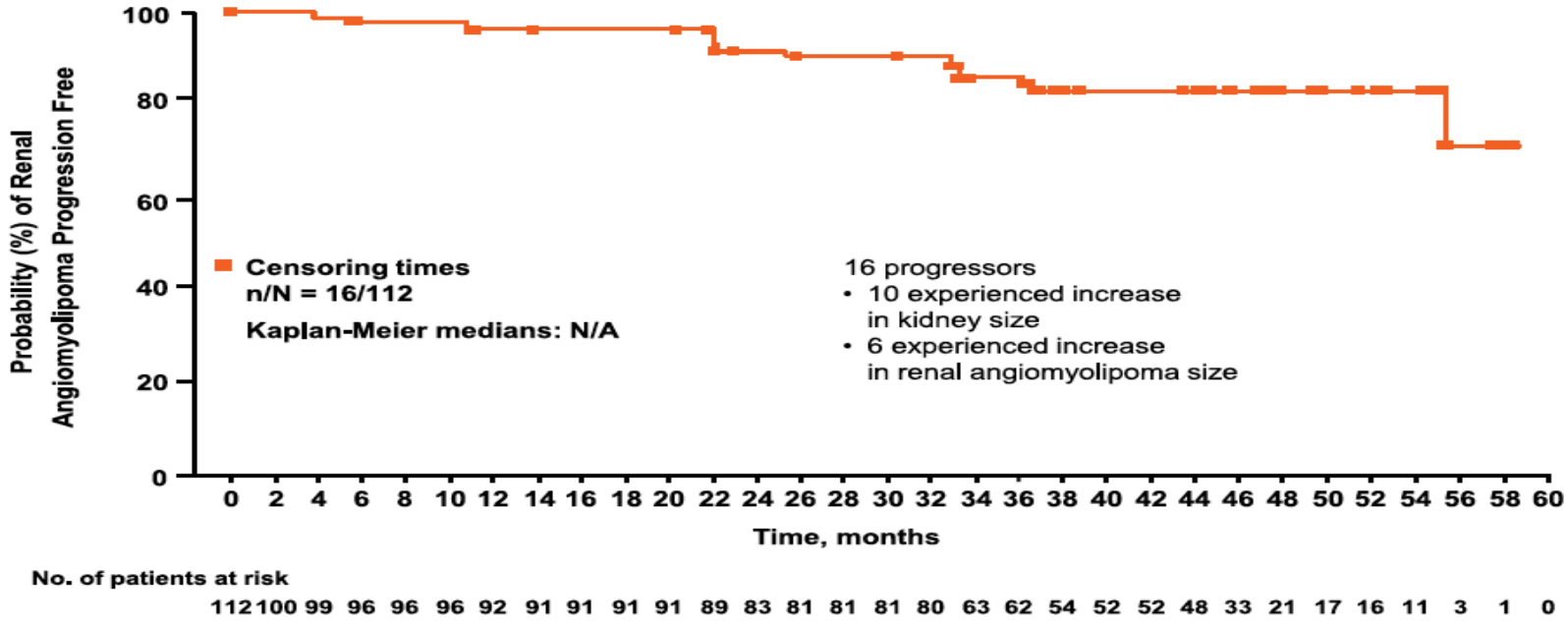
Bissler et al 2014

EXIST-2 EXTENSION: % reduction AML



Bissler et al PLOS one 2017

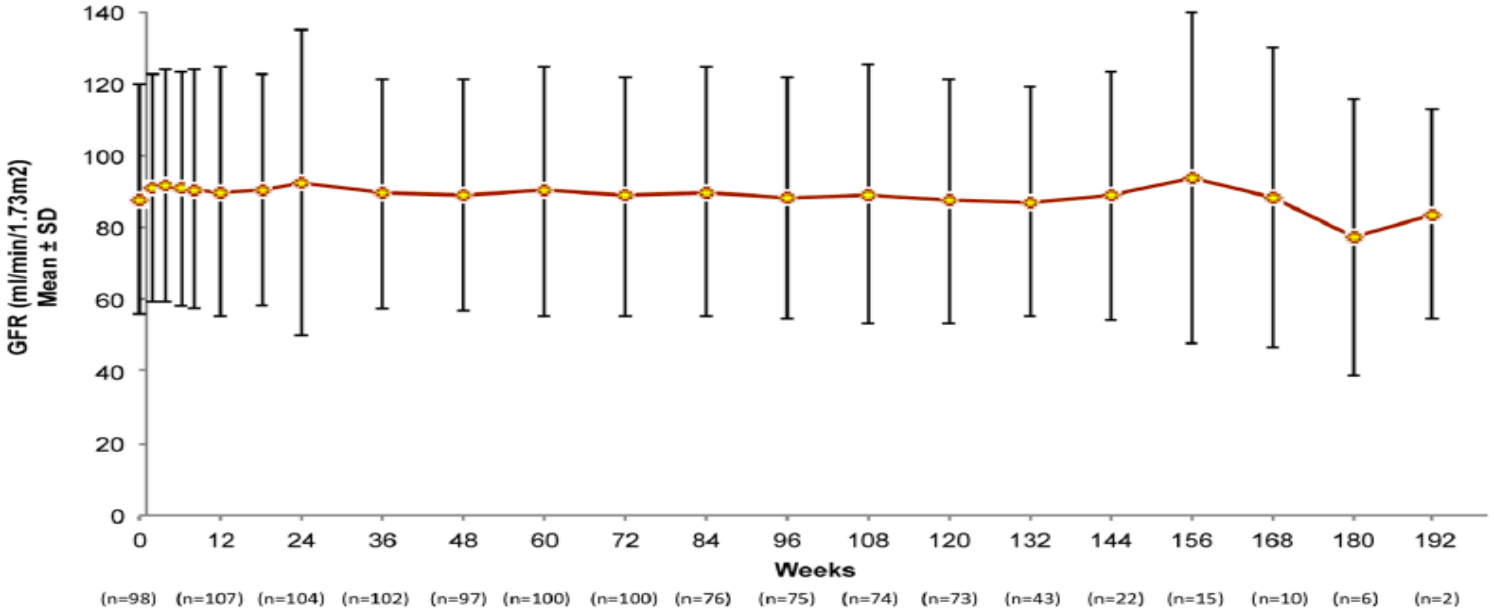
EXIST-2 EXTENSION: time to AML progression



NO BLEEDING

Bissler et al PLOS one 2017

EXIST 2 CKD



N=112

Bissler et al 2014

EXIST-2: do everolimus plasma levels correlate with efficacy?

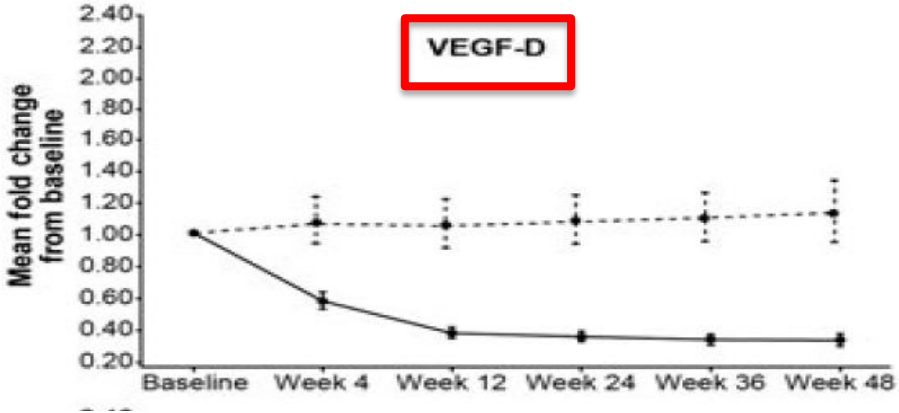
- EXIST-2: 10 mg per day. Only decrease because of AE. No modifications based on plasma levels.
- Percent change, rather than absolute change, from baseline in angiomyolipoma lesion volume was correlated with everolimus Cmin concentration
- For nephrologists: everolimus without plasma levels? PROBABLY NOT.
  - Suggested: 4-10 ng ml^{-1}

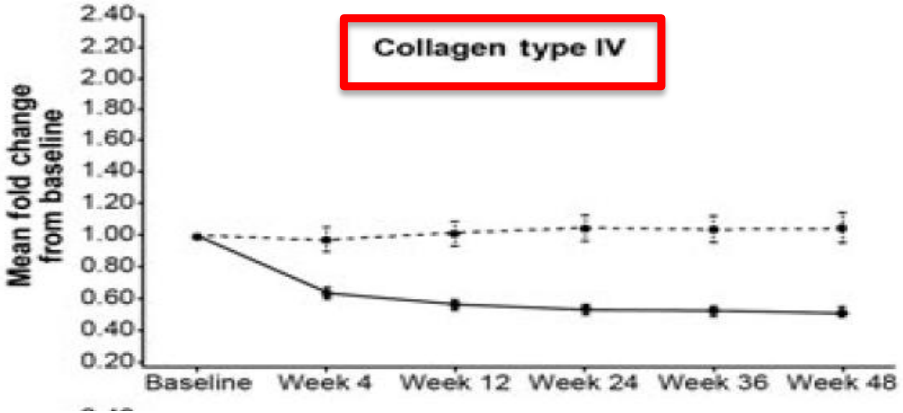
Budde et al Br J Pharmacol 2015

EXIST-2: Angiogenic biomarkers

placebo
everolimus

EXIST-2



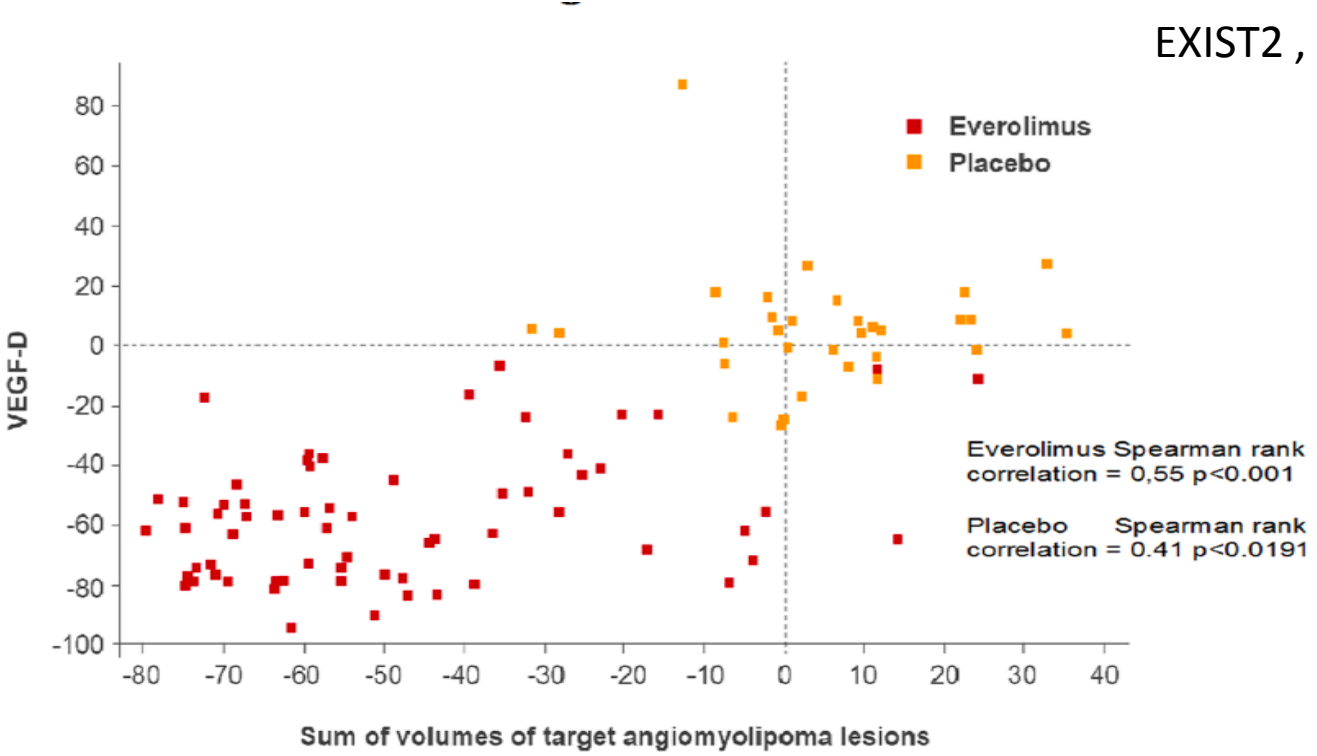


Moderate decrease in sVEGFR2 level and lack of everolimus effect on sVEGFR1, c-Kit and PLGF levels supports the hypothesis that everolimus may, at least partially, act through an anti-angiogenic mechanism in these patients

Budde et al Br J Pharmacol 2015

VEGF-D vs change of AML volume

EXIST2 , week 24



Bissler et al 2013

EXIST-2 EXTENSION: AEs

Adverse events, n (%)	≤12 months N = 112	13–24 months n = 101	25–36 months n = 100	37–48 months n = 91	49–60 months n = 52
Stomatitis	46 (41.1)	9 (8.9)	5 (5.0)	5 (5.5)	2 (3.8)
Nasopharyngitis	36 (32.1)	21 (20.8)	20 (20.0)	20 (22.0)	6 (11.5)
Acne	28 (25.0)	8 (7.9)	6 (6.0)	2 (2.2)	0
Headache	26 (23.2)	11 (10.9)	6 (6.0)	4 (4.4)	1 (1.9)
Hypercholesterolemia	25 (22.3)	13 (12.9)	11 (11.0)	7 (7.7)	1 (1.9)
Aphthous stomatitis	21 (18.8)	15 (14.9)	9 (9.0)	5 (5.5)	2 (3.8)
Fatigue	19 (17.0)	2 (2.0)	4 (4.0)	4 (4.4)	2 (3.8)
Cough	18 (16.1)	4 (4.0)	4 (4.0)	3 (3.3)	0
Diarrhoea	17 (15.2)	7 (6.9)	7 (7.0)	4 (4.4)	1 (1.9)
Mouth ulceration	17 (15.2)	6 (5.9)	5 (5.0)	2 (2.2)	0
Nausea	17 (15.2)	5 (5.0)	2 (2.0)	3 (3.3)	0

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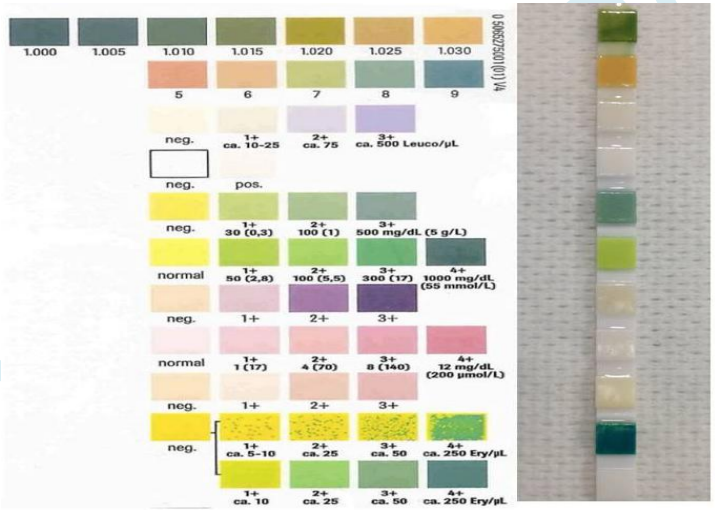
Bissler et al PLOS one 2017

EXIST-2 EXTENSION: AEs

- Stomatitis/mucositis/mouth ulceration (~ 50%)
- Hypercholesterolemia(20–40%)
- Hypertriglyceridemia (12–50%)
- Infections (40–70%)
- Hypophosphatemia (11%)
- Amenorrhea (13–38%)
- Hematologic abnormalities (microcytosis,leukopenia, neutropenia) (10–40%)
- Proteinuria/microalbuminuria (4–30%)

EXIST-2 EXTENSION: renal AEs

- eGFR declined if CKD was present at baseline
- What about proteinuria???



But microalbuminuria increases…

Patients	baseline creatinine (mg/dL)/MDRD (ml/min/1.73m2)	24 month creatinine (mg/dL)/ MDRD (ml/min/1.73m2)	baseline proteinuria[1] (mg/mmol)	24 month proteinuria[1] (mg/mmol)	baseline cholesterol/ HDL/LDL (mmol/L)	24 month cholesterol/ HDL/LDL (mmol/L)	baseline triglycerides (mmol/L)	24 month triglycerides (mmol/L)
1	0.84 /76	0.78/81	6.1	12.1	159/89/90	184/74/101	45	52
2	0.93/>90	0.95/>90	22.4	40.0 [5]	118/46/72	167/54/100	43	63
3	1.22/74		22.5	[2]	205/74/131		86	
4	0.96/71		10.3	[3]	176/83/94	[3]	144	[3]
5	0.99/87	0.97/88	9.1	8.2	202/63/139	181/44/125 [4]	116	64
6	0.67/>90	0.58/>90	5.0	9.6	240/86/156	152/63/89 [4]	198	116
7	1.15/50 [0]	0.98/60	9.4	28.6 [5]	192/78/114	188/77/102	47	47
8	1.07/83	1.09/80	5.6	4.0	154/56/98	202/66/120	62	77
9	0.77/>90	0.86/>90	13.2	47.0 [5]	125/36/90	226/52/136 [4]	102	186
10	0.85/78	0.77/87	5.0	4.3	212/55/157	167/75/82 [4]	48	51
11	0.71/>90 [0]	0.86/78	7.7	9.4	94/40/54	118/41/64	76	62
12	0.42/> 90	0.48/>90	13.3	11.1	142/38/104	193/42/127	117	120
13	0.62/>90	0.61/>90	6.6	9.8	183/96/87	163/84/90	53	41
14	0.83/>90	1.01/>90	6.4	8.9	203/83/120	176/39/104 [4]	175	163
15	0.68/>90	0.69/>90	9.0	18.2	200/78/122	170/75/80	66	73
16	0.62/>90	0.54/>90	12.9	11.9	156/51/105	120/49/50 [4]	145	104
17	1.30/42 [0]	1.24/44	22.4	32.3 [5]	292/106/189	216/69/147 [4]	243	117

0-Patients 7, 11, 17 had undergone a nephrectomy at least one year before the start of the trial
1-Expressed as a protein-to-creatinine ratio
2-Patient 3 was withdrawn at 12 months of treatment due to nephrotic-range proteinuria that reverted after discontinuation of treatment.
3-Patient 4 was excluded at 10 months due to acute pyelonephritis and did not want to be rechallenged.
4-Statins were prescribed in patients 5, 6, 9, 10, 14, 16, 17
5- ACEI were prescribed for microalbuminuria in patients 2, 7, 9, 17

1 patient in EXIST-2 and one in Barcelona trial: **nephrotic range proteinuria**

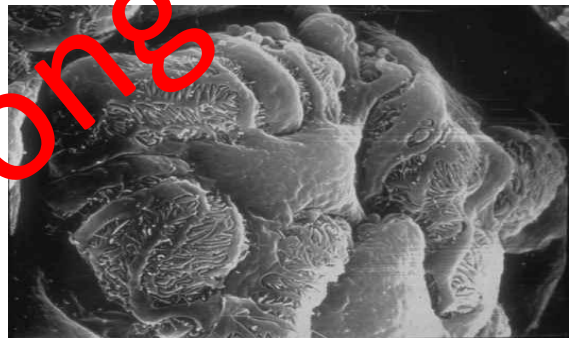
Cabrera et al OJRD 2012

LONG TERM EFFECTS OF mTOR inh IN THE KIDNEY

- Podocitary expression of nephrin, TRPC6 and Nck are significantly decreased under long term mTOR inhibitors exposure
- mTOR inhibitors reduce podocitary adhesion and motility

- Long term effects on proteinuria and kindey function are unknown

Life long treatment!!



Then…

- Will mTOR inhibitors target several renal abnormalities in TSC kindeys

or

- Will they worsen the progression of CKD?

Everolimus for other TSC manifestations

Subependimal giant cell astrocytoma. EXIST-1

EUROPEAN MEDICINES AGENCY

Epilepsy. EXIST-3

EUROPEAN MEDICINES AGENCY

LAM

Facial angiofibromas

Surveillance and management recommendations of the International TSC Consensus Group

Newly diagnosed or suspected TSC	Diagnosed with definite or possible TSC
Surveillance of kidneys	
Obtain MRI of the abdomen to assess for the presence of angiomyolipoma and renal cysts	Obtain MRI of the abdomen to assess angiomyolipoma progression and renal cystic disease (every 1–3 years for life)
Screen for hypertension by obtaining accurate blood pressure	Assess renal function (GFR and blood pressure) at least annually
Evaluate renal function by determining GFR	
Clinical presentation	**Recommendation**
Management recommendations for renal angiomyolipoma	
Angiomyolipoma with acute hemorrhage	Embolization (followed by corticosteroids for 7 days to mitigate post-embolization syndrome) [3]. Embolization should be as selective as technically feasible to preserve renal parenchyma Avoid nephrectomy
Asymptomatic, growing angiomyolipoma >3 cm in diameter	First-line: mTOR inhibitor Second-line: selective embolization or kidney-sparing resection

Kingswood et al, Nephron 2016;134:51–58

Conclusions-future directions

- mTOR inhibitors: first choice for preemptive treatment of growing AML >3 cm in diameter
- Potential benefits of preventive therapy in reducing AML-related morbidities may outweigh the risks of long-term therapy

- Future studies should address the impact of early detection and appropriate treatment of renal AML on preserving renal function (before AML>3 cm?)
- Plasma angiogenic biomarkers as measure of treatment efficacy
- Future studies should address the impact of adverse events related to mTOR.

Sant Pau

Fundació Puigvert

Thanks!!!

Next webinar: June 29, Claus Schmitt (Heidelberg)

Optimizing PD in Children