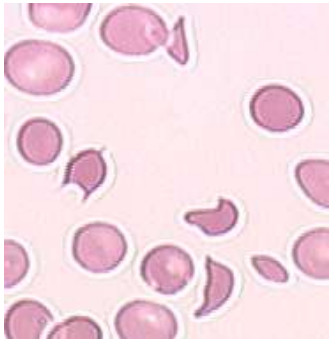
Thrombotic microangiopathies and antineoplastic agents



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Actualités Néphrologiques Jean Hamburger – hôpital Necker – 25 avril 2017

THROMBOTIC MICROANGIOPATHIES

- Microangiopathic hemolytic anemia
- Peripheral thrombocytopenia
- Organ failure of variable severity

TTP

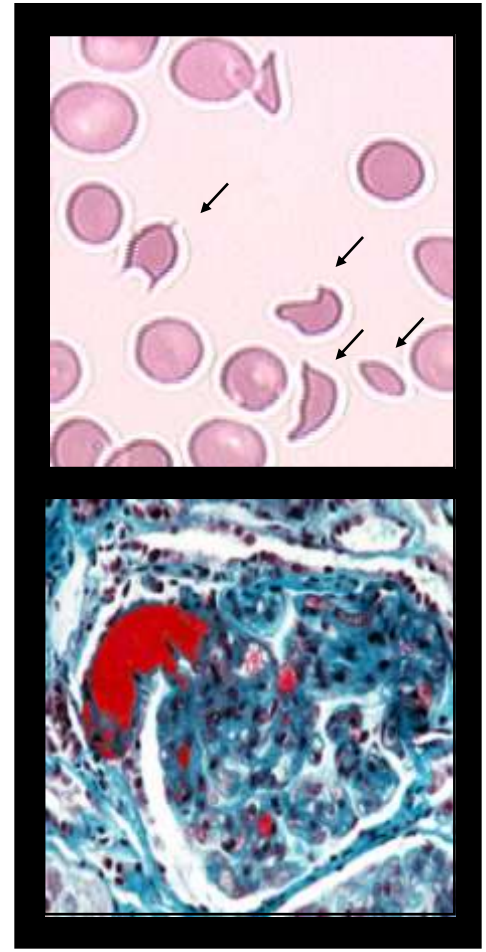
- Congenital
- Autoimmune
  - Idiopathic
  - Secondary

HUS

- STEC+
- Complement+

Other entities

- HELLP Sd
- HTA malignes
- Transplantation
- Cancers
- Antineoplastic agts



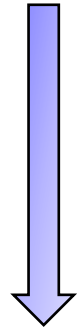
Epidemiology: burden of patients

Proportion of chemo-associated TTP (from 772 TTP patients)

	Number of patients (proportion of 772 TTP)
Infections	94 (12%)
AI diseases	87 (11.5%)
Cancer	71 (9%)
Pregnancy	62 (8%)
Transplantation	27 (3.5%)
HIV	24 (3%)
Other / several conditions	18 (2,5%)
Drugs	11 (1.5%)

≈ 50%

> 100 chemo-associated TMA in the French TMA registry



> 90% of patients have a detectable activity

Most chemo-associated TMA have a detectable ADAMTS13 activity (= not TTP)

Mariotte et al., Lancet Haematol 2016 and unpublished results

Antineoplastic drug-associated TMA

Medina et al., Curr Op Hematol 2001

Moll et al., Am J Kidney Dis 2001

Eremina et al., N Engl J Med 2008

Yui et al., Am J hematol 2016

Many have been associated with TMA:

- Mitomycine C
- Gemcitabine
- Anti-VEGF
- Proteasome inhibitors (carfilzomib, bortezomib)
- $Yttrium^{90}$ (> 200 mCi/m^2: associated with renal TMA)
  Anti-CD22 immunotoxin (BL22)
- Tyrosine kinase inhibitors (imatinib mesylate, dasatinib)
- Deoxycoformycine (Nipent®)

  Cytarabine
  Daunorubicine
  Cisplatine

] Exceptionally reported…

Mitomycin C-associated TMA

Medina et al., Curr Op Hematol 2001

Table 3. Clinical characteristics of patients with mitomycin C–associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome

	Lesesne [11]*	Snyder [21]*	Sheldon [12]*	Cantrell [17]*
Number of patients	85	55	39	12
Chemotherapy regimen included mitomycin C	99	93	82	100
Cumulative dose of mitomycin C >40 mg	99	NR	NR	100
Sex (% female)	59	78	59	58
Primary site of carcinoma				
gastric	26	9	44	50
breast	18	44	9	8
colorectal	16	22	20	8
Clinical features				
pulmonary	65	NR	49	100
neurologic	16	NR	18	25
Laboratory features				
microangiopathic hemolytic anemia	100	95	90	100
thrombocytopenia	100	78	92	100
renal failure	100	78	92	100
Death	74	55	72	83

*All values except number of patients are percentages.
NR, not recorded.

TMA in 2% to 15% of patients receiving MMC

Clinical features typically occur 4 to 8 weeks after the last MMC infusion

Usual cumulated dose > 40 mg

Lung involvement is a frequent feature+++

- **Dyspnea**
- **Lung oedema**
- **Respiratory distress**

Renal failure if cumulated dose > 50-70 mg

ADAMTS13: normal or mildly decreased

Diffuse endothelial lesions induced by the drug

Poor response to plasma exchange ± immunoadsorption

Poor prognosis; death at ~ 4 months

Gemcitabine-associated TMA

Wasif Saif & McGee, 2005

TMA in ~ 15/100 000 treated patients

Median duration of treatment: 5.8 m

Occurs 1-2 months following the last infusion

TMA syndrome with renal failure and HT

ADAMTS13 detectable or normal

Features	Idiopathic TTP	Gemcitabine-associated TTP	References
Incidence	3–7/million	150–14 000/million	2
Sex distribution, F:M	3:1	1:1	18,20–25,34
Hypertension, %	NA	74	18,20–25
Neurological symptoms, %	50	NA	35
Pulmonary symptoms, %	NA	60	20,22–24
Renal insufficiency*, %	45	100	18–23,26,34,36,37
Schistocytes, %	90	88	18,20,21,24,38
ADAMTS-13 deficiency†, %	33–100	13	39,40
Mortality, %	10	13	10,18,20,22–25,41

*Renal insufficiency defined as creatinine ≥133 μmol/L. †ADAMTS-13 activity <5%. F=female; M=male. NA=not available. ADAMTS-13=A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13

Pathophysiology: unclear+++

- « Endothelial injury »?

- No clear threshold of toxicity (20 g/m^2 for some authors); « immunologic » mechanism (IC) ?

Response to plasma exchange typically poor; immunoadsorption ? Treat HT; dialysis; stop TTT

Mortality ranges from 10% to 70% of cases (variable; role of the underlying disease?)

Gemcitabine-associated TMA: therapy with complement blockers

Eculizumab therapy for gemcitabine induced hemolytic uremic syndrome: case series and concise review

Omar Al Ustwani[1], James Lohr[1], Grace Dy[1], Charles LeVea[2], Gregory Connolly[3], Pradeep Arora[1], Renuka Iyer[1]

4 patients with gemcitabine-associated TMA:

	Age	Cancer	HT	A13	Creatinine	Eculizumab
1	75	Stage IV squamous carcinoma of the lung	+++	74%	2.6 mg/dl	Improved
2	70	Metastatic pancreatic carcinoma	NA	79%	1.82 mg/dl	Improved
3	73	Metastatic cholangiocarcinoma	NA	NA	3.79 mg/dl	Stabilization; subsequently died
4	69	Stage IV squamous carcinoma of the lung	NA	>95%	2.79 mg/dl	Improved

Median cumulated dose: 21.2 g/m2

Resolution of TMA features

Improvement of renal function though no recovery

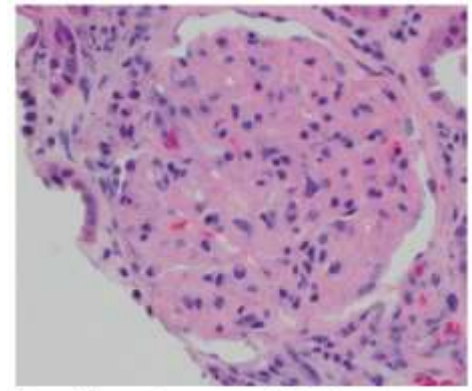
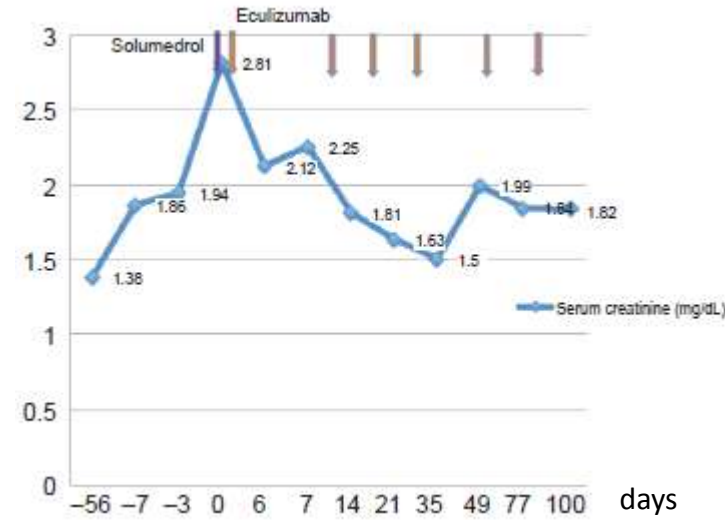


Figure 1 Microscopic appearance of kidney biopsy from patient 1 (×400), showing diffuse segmental thickening of glomerular basement membranes and increased extracellular matrix within the glomeruli. The findings are consistent with thrombotic microangiopathy/antineoplastic drug-induced atypical hemolytic uremic syndrome.



J Gastrointest Oncol 2014

Gemcitabine-associated TMA and eculizumab: the French experience

- **Observational, retrospective study including all patients with gemcitabine-induced TMA treated by eculizumab in 4 French centres, between 2011 and 2015**

- **6 women, 2 men**

  Gemcitabine for pancreatic (n=3, 37.5%), ovarian (n=3, 37.5%) and pulmonary (n=2, 25%) cancer

  TMA occurred after a median of 5 months (range 1.7-13) following treatment initiation

  Median cumulative dose of 27.5 g (range 0.9-48.0)

- **Hemolytic anemia (100%), acute renal failure (100%; including 62% stage 3 AKI and 25% dialysis)**

  Hypertension (75%)

- **Eculizumab was started after a median of 19.5 days (range 6-44) following TMA diagnosis**

  A median of 4.5 injections of eculizumab was performed (range 3-22)

Grall et al., in preparation

Gemcitabine-associated TMA and eculizumab: the French experience

- **Major hematologic response in 6 patients (75%)**

  RBC transfusion decreased after 1 injection of eculizumab: median of 2 packed RBC (0-10) vs 0 (0-1)

- **Complete recovery of renal function was achieved in 2 patients (25%)**

  Partial response in 4 patients (50%) with a median improvement of 15 ml/min/1.73m^2 (range 7-16) estimated GFR

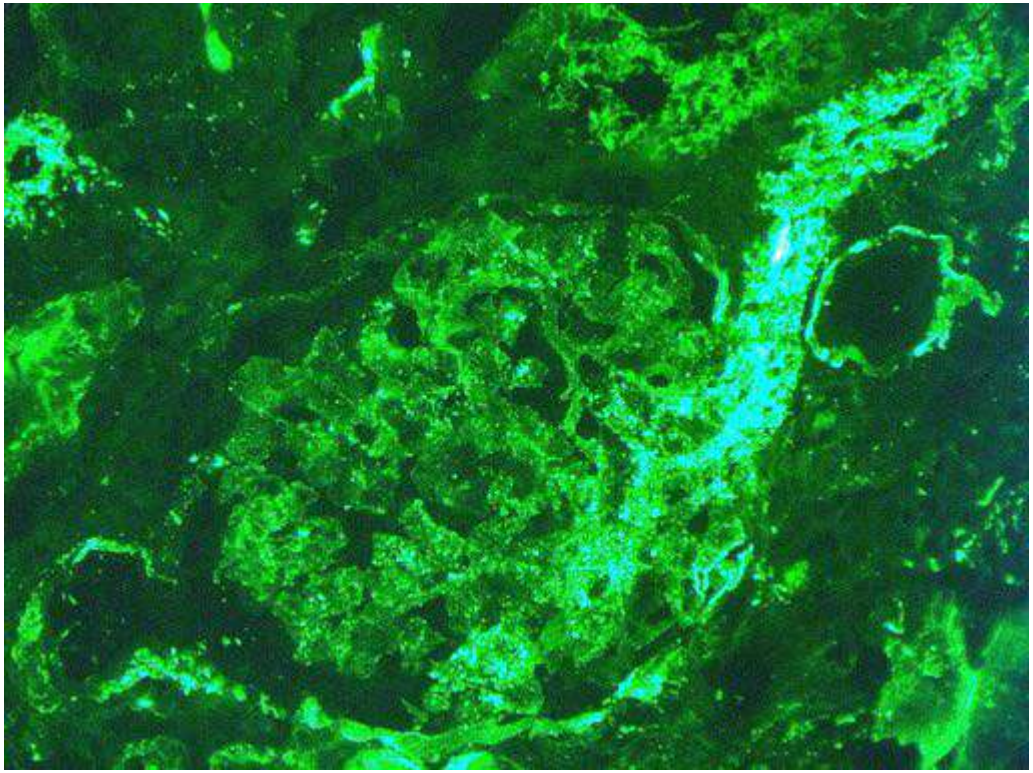
- **Five patients (62.5%) died during follow up despite response (septic and hemorrhagic shock on early stage: 1, cancer evolution after a median of 6 months (range 2-13) following eculizumab initiation: 4)**

Acceptable indication in gemcitabine-associated TMA provided a reasonable underlying oncologic prognosis

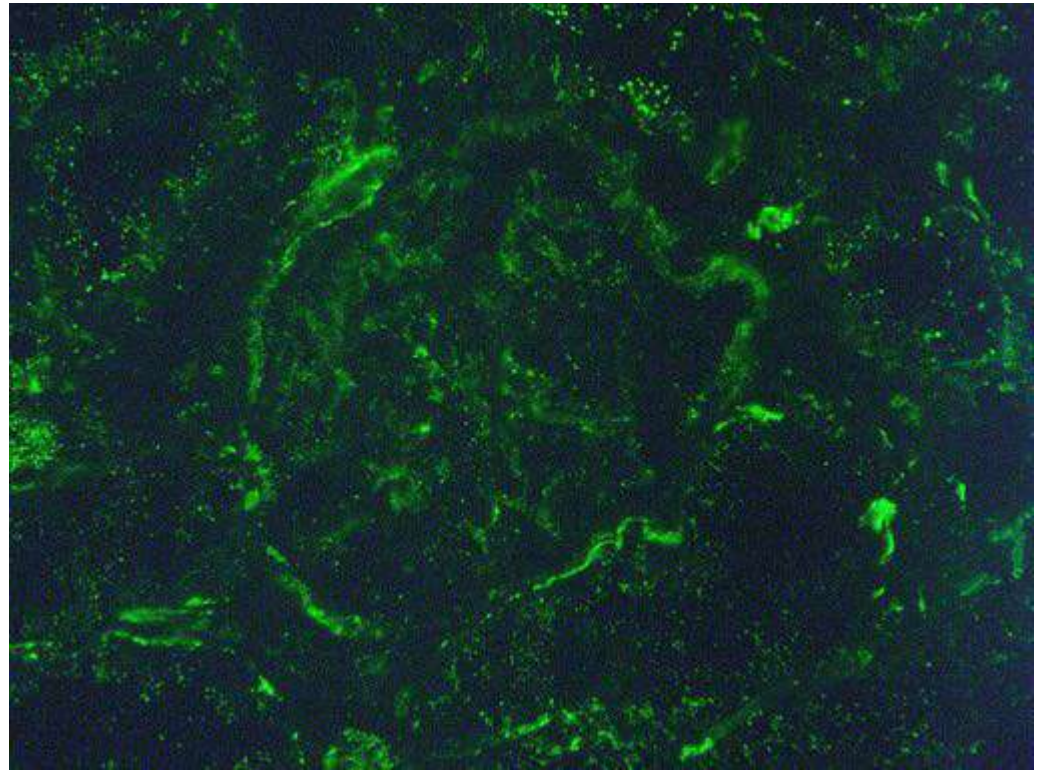
Grall et al., in preparation

Possible role of complement in gemcitabine-induced TMA

Immunostaining with anti-C5b9 (MAC)



Patients (N=3)



Control

Positive staining in glomerular and tubular membrane and also in the capillary wall

Grall et al., in preparation

TMA and anti-VEGF agents

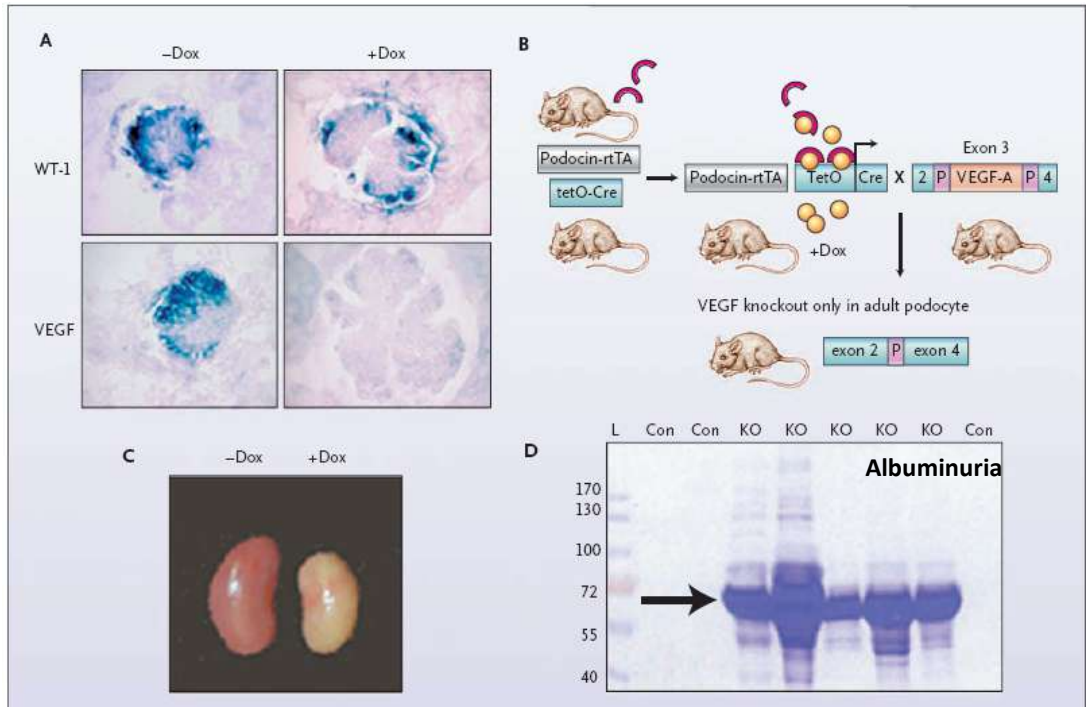
VEGF inhibition leads to TMA

6 patients treated with bevacizumab developed a TMA syndrome (proteinuria, hypertension, TMA)

VEGF has a role in vascular homeostasis

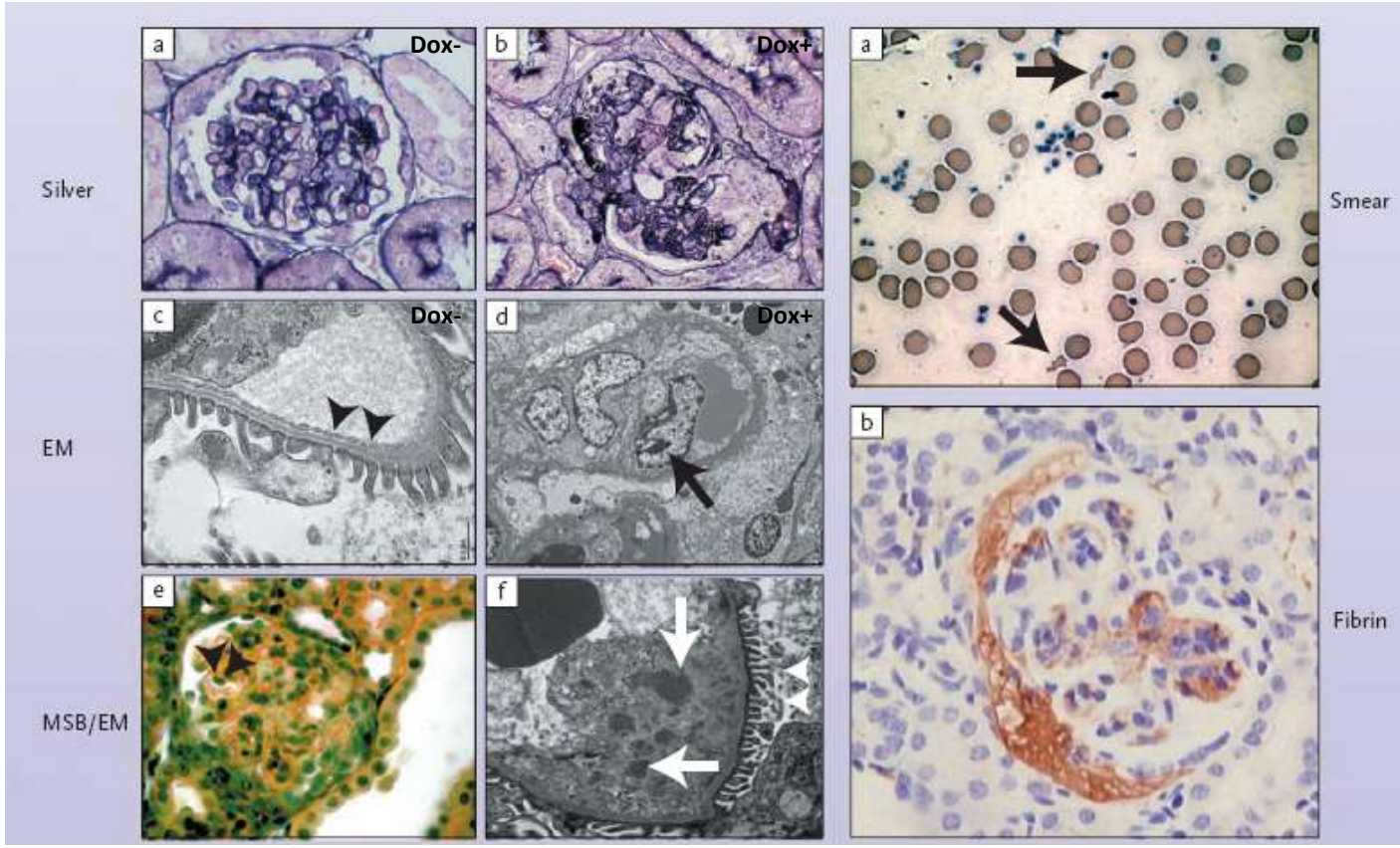
Question: is VEGF inhibition sufficient to induce a TMA?

Murine model of TMA by VEGF inhibition: VEGF KO tissue (podocyte)-specific and Dox+ inductible



Eremina et al., N Engl J Med 2008

VEGF has a major role in vascular homeostasis



Schistocytes on blood smear

IHC positive for fibrin

Lumens of glomerular capillaries are collapsed or obliterated (b)

Endothelial cells swollen (d); dense subendothelial deposits (f)

Intracapillary thrombi (e)

Eremina et al., N Engl J Med 2008

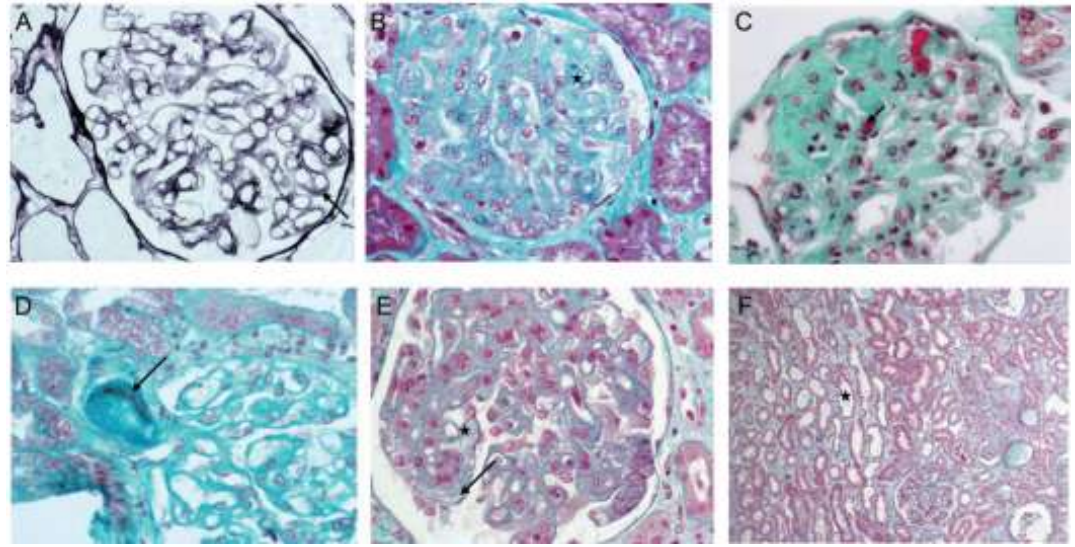
Anti-VEGF agents and pre-eclampsia-like syndrome

22 patients – Renal biopsy 16.2 ± 10.6 months > anti-VEGF (MoAb, TKI and VEGF-Trap) initiation for proteinuria or renal failure

Table 1. Clinical characteristics of the 22 patients who had a renal biopsy for proteinuria and/or renal failure dur

No. of patient	Sex	Age	Cancer localization	Anti-angiogenic drug	RAS blocker	Serum creatinine (μmol/L)
1	M	56	Rectum	Bevacizumab	ACEi	80
2	M	71	Liver	Brivanib then nexavar	ARB	153
3	F	40	Breast	Bevacizumab	ACEi	56
4	F	52	Breast	Bevacizumab	ARB	Anuria
5	F	64	Kidney	Sorafenib then sunitinib	ACEi	124
6	M	48	Kidney	Bevacizumab	ARB + ACE	117
7	M	75	Liver	Sorafenib or brivanib (phase III study)	ARB	66
8	M	70	Kidney	Sunitinib	ARB	400
9	M	71	Rectum, liver and lung metastasis	Bevacizumab	ACEi	100
10	M	64	Lung	Aflibercept	ARB	89
11	M	57	Liver	Bribanib	ACEi	63
12	M	60	Caecum	Bevacizumab	ACEi	92
13	F	56	Pancreas	Sunitinib	ACEi	290
14	F	46	Breast	Bevacizumab	ACEi	69
15	F	47	Breast	Bevacizumab	ACEi	64
16	F	61	Glioblastoma	Bevacizumab	ACEi	62
17	F	43	Breast	Bevacizumab	ARB	62
18	M	80	GIST	Sunitinib	ARB + DRI	220
19	F	47	Breast	Bevacizumab	0	60
20	M	64	Pleura	Bevacizumab	ACE-i	135
21	F	68	Breast	Bevacizumab	ACE-i	80
22	M	55	Glioblastoma	Bevacizumab	No	72
Mean (±SD)	12 M/10 F	59 ± 11			20/22	134 ± 117

ACEi, ACE inhibitor; ARB, angiotensin receptor blocker; DRI, direct renin inhibitor; MPGN, membrano-proliferative glomerulo nephi

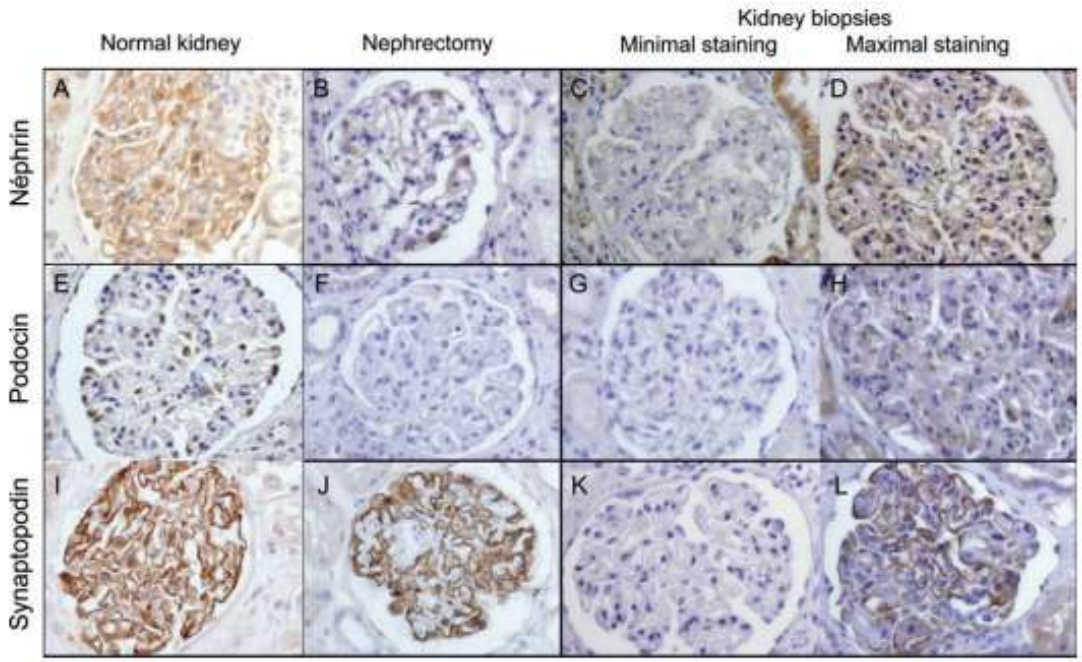


Double contours+thrombi of capillaries, duplication of the glomerular basement membranes, widening of the subendothelial spaces, endothelial swelling, consistent with features of TMA

Hypertension in ~ all cases –Mean proteinuria 2.97 ± 2.0 g/day - No or mild signs of TMA
Renal failure variable (normal creatinine level to 400 mcm.L^{-1}/anuria)

Vigneau et al., Nephrol Dial Transplant 2014; Izzedine et al., Medicine 2014

Down-regulation of podocyte proteins with anti-VEGF agents

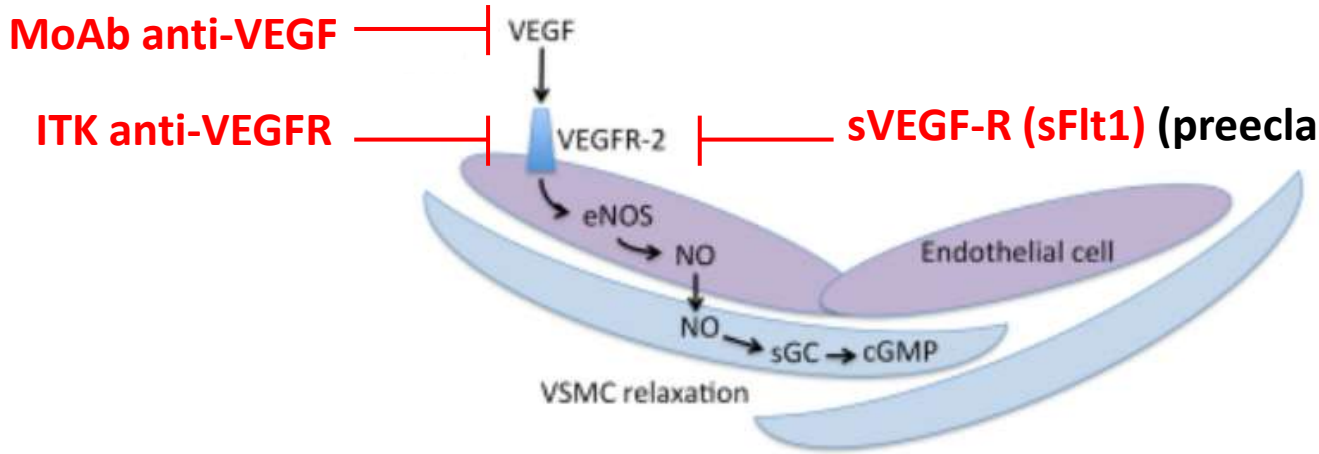


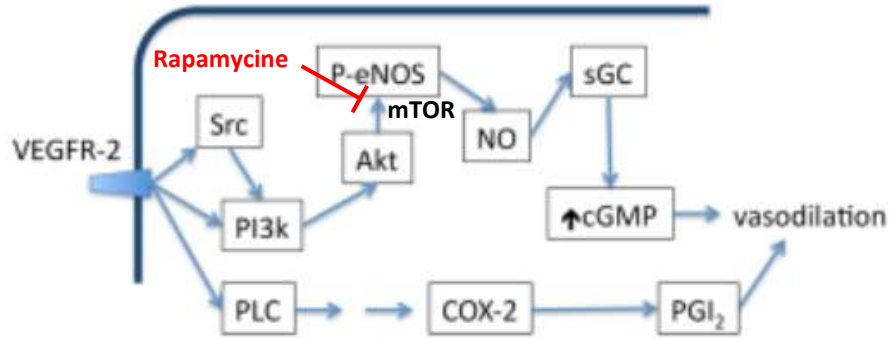
No. of patient	Number of glomeruli	Podocin	Nephrin	Synaptopodin	VEGF	ATN (%)
1	14	0/+	0	+	0/+	0
2	27	0	++	++	1	0
3	5	0	0	0/+	NA	>50
4	20	0	0	NA	?NA	0
5	10	0	+	0	NA	0
6	5	+	+	0	+	15
7	10	+	0/+	+	NA	30
8	7	0	+	+/++	ND	0
9	7	0	+	+	0/+	0
10	13	0	++	0/+	0/+	20
11	5	0	0	0/+	0/+	10
12	13	0	0	0	0	30
13	11	0	0	0/+	0/+	<10
14	13	0	++	++	0	0
15	27	0	++	++	0/+	0
16	10	0	+	+	0/+	40
17	14	0	0/+	+	0/+	0
18	22	0	0	0	0/+	<10
19	12	0	0	+	0	0
20	10	0	0	0	0/+	<20
21	10	0	0/+	+	0/+	0
22	16	0	0/+	0	0/+	<10

Nephrin, podocin, synaptopodin are down-regulated, as a possible consequence of VEGF inhibition and may lead to features of pre-eclampsia

Vigneau et al., Nephrol Dial Transplant 2014

Role of VEGF pathway in vascular homeostasis: a general theme in TMA





- VEGF/VEGFR (/AKT/mTOR/eNOS) pathway has a major role in vascular homeostasis (kidney, placenta)
- VEGF induces the formation of fenestrations in endothelium; loss of VEGF = microvascular injury & TMA

Robinson et al., Semin Nephrol 2010

Proteasome inhibitor-associated TMA

TABLE I. Laboratory Values at TMA Diagnosis and Clinical Manifestations.

	Age and sex	PI used	Timing[a]	Hgb (g dL^{-1})	Platelet count, ×109/L	Cr (mg dL^{-1})	LDH (U L^{-1})	Hapto (mg dL^{-1})	ADAMTS13 activity	Dialysis required	TMA on renal biopsy	AST (U L^{-1})	GI sx	Neuro sx
1	70 M	Bortezomib	21 d	6.9	66	9.9	631	<14		Y		50	Y	N
2	64 M	Bortezomib	9 d	9.2	17	0.8	659	<14		N		118	N	N
3	51 M	Bortezomib	21 d	7.5	119	2.65	218	<2	34%	Y	Y	49	N	N
4	80 M	Carfilzomib	5 d	11.2	11	6.1	1920	<14	100%	Y		96	N	Y
5	79 M	Carfilzomib	8 mo	8.4	18	7.29	3481			Y		137	Y	Y
6	67 M	Carfilzomib	17 mo	10.3	20	3.12	642			N		43	N	N
7	64 F	Carfilzomib	8 mo	11.9	8	1.1	1848	<10	88%	N		123	Y	N
8	67 F	Carfilzomib	7 d	7.3	34	8.1	698	<8	79%	Y		36	Y	N
9	45 M	Carfilzomib	6 mo	4.6	163[b]	1.75	250	34		N		17	Y	Y
10	44 M	Carfilzomib	8 mo	6.7	39	7.28	1220	3		N	Y	58	Y	N
11	49 M	Carfilzomib	6 d	7.2	18	2.4	1129	<14	82%	N		36	N	N

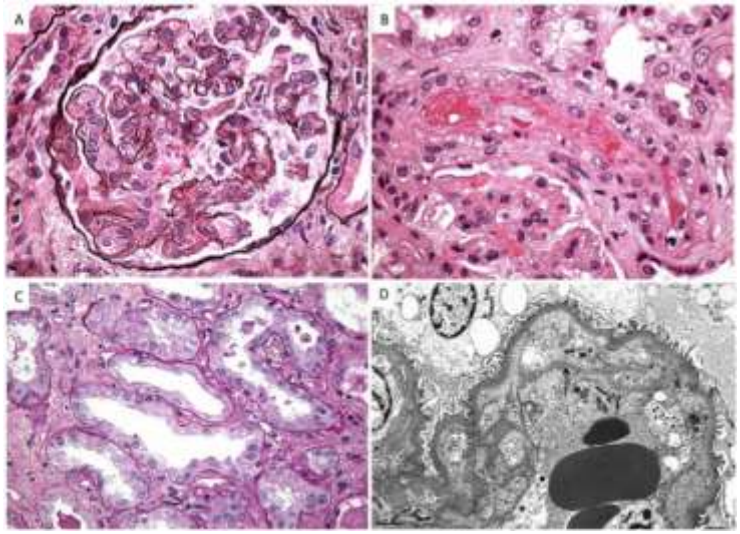
Bortezomib n=3
Carfilzomib n=8

Variable timing

ADAMTS13 detectable

5/11: dialysed/CNS rare

Liver/digestive tract+++



TPE: n=4; eculizumab: n=3

9/11: resolution of TMA; 2/11: stabilization of MAHA

1 patient: recurrence of TMA after PI reintroduction

3 deaths, including 1 responder

Strong level of evidence that PI can cause TMA

Yui et al., Am J hematol 2016

How to distinguish antineoplastic drug-associated TMA from paraneoplastic TMA?

Cancer-associated TMA: clinical features

Oberic et al., Oncologist 2009

Table 1. Clinical findings

Patient no.	Sex	Age (years)	Type of cancer	Metastasis	Symptom duration before TMA diagnosis	Weakness associated with weight loss and anorexia	Fever	Bone pain	Dyspnea	Abdominal pain	Thoracic pain	CNS involvement[a]
1	F	74	Lung	Bone	30 days	Y	N	Y	Y	N	Y	N
2	F	54	Colon	Bone; bone marrow; lymph nodes	30 days	Y	N	Y	Y	N	N	Y
3	F	70	Breast	Bone; bone marrow	3 days	N	Y	N	N	N	N	Y
4	F	55	Breast	Liver; pleural; bone	6 days	Y	Y	N	N	Y	N	Y
5	F	65	Breast	Liver; bone marrow	30 days	Y	N	N	N	N	N	Y
6	F	51	Breast	Bone marrow	10 days	N	N	N	N	N	N	N
7	F	38	Stomach	Bone marrow; lymph nodes	30 days	Y	N	N	N	Y	N	N
8	F	75	Breast	Lung	30 days	Y	N	Y	Y	N	N	Y
9	M	52	Stomach	Bone; bone marrow; liver	15 days	Y	Y	Y	Y	N	Y	N
10	M	51	Stomach	Bone; lung	15 days	Y	N	Y	Y	Y	Y	N
11	F	59	Stomach	Pleura; bone	5 days	Y	N	Y	Y	N	N	N
12	M	79	ACUP	Bone; bone marrow; lymph nodes	30 days	Y	N	Y	Y	N	N	N
13	F	60	Liver	Lymph nodes; bone marrow	190 days	Y	N	N	Y	N	N	N
14	M	69	Prostate	Bone	1 day	N	N	Y	N	Y	N	N
15	F	53	Lung	Liver	7 days	Y	N	Y	Y	N	N	N
16	M	74	Liver	Bone; bone marrow; lymph nodes	180 days	N	N	Y	N	N	N	Y
17	M	65	Prostate	Bone; lung; meninges	NA	Y	Y	N	N	N	N	Y
18	F	64	Breast	Bone; liver	60	Y	Y	N	N	N	N	N
19	F	38	Breast	Bone; bone marrow	85	N	N	N	Y	N	N	Y
20	M	66	Lung	Brain	19	Y	N	N	Y	N	N	Y

Breast: 7 cases

Stomach: 4 cases

Lung: 3 cases

Liver: 2 cases

Colon: 1 case

Prostate: 2 cases

Indetermined: 1 case

Wasting, weight loss: 75%

Multimetastatic cancer

Bone pain: ~ 50%

Cancer-associated TMA: clinical features

Oberic et al., Oncologist 2009

Table 2. Biological findings

Patient no.	ADAMTS13 activity (%)	ADAMTS13 inhibitor[a]	Hemoglobin level (g/dl)	Reticulocyte count (10^3/mm^3)	Schistocytes	LDH (×N)	Platelet count (10^3/μl)	Prothrombin rate (%)	Fibrinogen level (g/l)	D-dimers (μg/ml)	Creatinine level (μM)	Estimated GFR (ml/minute)
1	49	—	7	164	+	3.8	60	72	3	>4	50	70
2	118	—	8.7	142	+++	3.2	20	63	NA	NA	59	82
3	NA	NA	6.4	295	+	4.5	116	80	1	>4	110	30
4	41	—	5.5	331	+++	21.5	17	58	1.7	>4	80	54
5	NA	NA	13	258	++	7.8	21	57	2.1	NA	71	50
6	64	—	9.1	213	+++	2.5	45	85	2.8	>4	72	83
7	28	—	7.5	144.5	+++	5.3	39	64	1.4	>4	62	103
8	58	—	9.3	196	+	8.9	190	84	NA	NA	94	44
9	21	—	6.8	146	++	6.9	18	72	2.7	>4	76	103
10	126	—	7.4	212	+++	3.3	74	75	0.9	>4	74	79
11	NA	NA	6.6	138	+++	18.1	39	57	3.4	NA	74	70
12	0	1	8.7	89	+	2.9	109	76	1.71	>4	129	39
13	0	1	9.2	NA	++	6.2	103	47	1.1	>4	51	110
14	20	0	10.4	50	++	NA	14	67	2.1	NA	636	10
15	129	—	6.9	343	++	3.7	50	77	3.4	NA	72	75
16	37	—	9.3	76	+	8.9	60	62	7	NA	343	16
17	0	0	9.2	337	++	3	35	74	2.7	>4	92	73
18	93	—	6.8	NA	+	3.6	58	69	8.3	>4	73	64
19	75	—	7.9	190	++	3.2	72	59	1.9	>4	65	101
20	57	—	11.7	220	++	14.3	21	68	4.3	NA	120	50
	39% (0–70)		8.3 (6.9 –9.3)	193 (143–259)		4.5 (3.2-8.9)	48 (21-73).				74 (68–102)	70 (48–82)

ADAMTS13 normal/detectable (82% of cases)

DIC common (Fg < 2 g/L in 40% of cases)

Renal involvement usually mild

Peripheral erythroblasts (85% of cases)

Bone marrow exploration

Oberic et al., Oncologist 2009

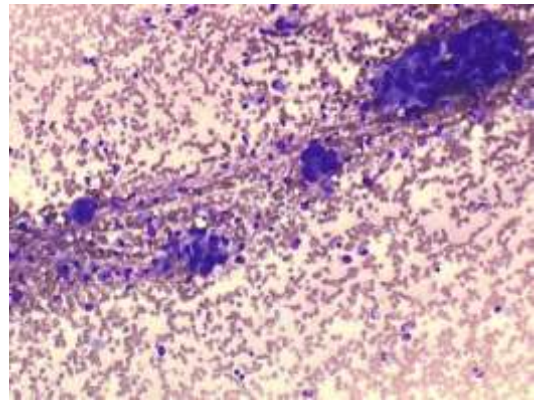
Table 3. Bone marrow aspiration and/or biopsy findings

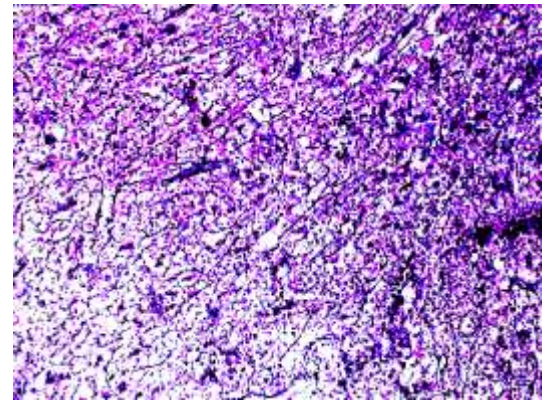
Patient no.	Results of bone marrow exploration
1	Erythroblastic hyperplasia
2	Metastatic cells; fibrosis
3	Metastatic cells
4	Metastatic cells
5	Metastatic cells
6	Metastatic cells
7	Metastatic cells
8	Erythroblastic hyperplasia
9	Metastatic cells; fibrosis
10	Metastatic cells
11	Erythroblastic hyperplasia
12	Metastatic cells; fibrosis
13	Metastatic cells; fibrosis
14	NA
15	NA
16	Metastatic cells
17	NA
18	NA
19	Metastatic cells
20	NA

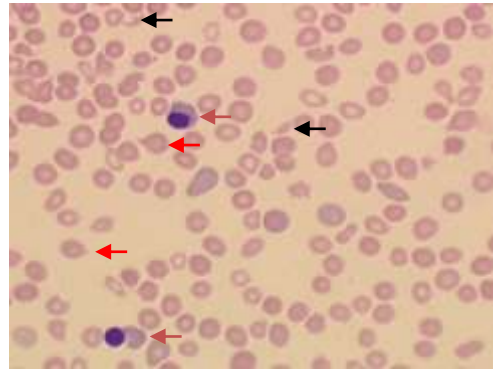
Abbreviation: NA, not available.

Bone marrow metastasis in 12/15 patients explored

Bone marrow fibrosis in 4 patients







- Schistocytes
- Tear drop cells
- Erythroblasts

How to distinguish antineoplastic drug-associated TMA from cancer-associated TMA

	Antineoplastic drug-associated TMA	Cancer-associated TMA
Wasting, weight loss, bone pain	0	+++
Hypertension	+++	0
Pulmonary symptoms	++	+
Renal insufficiency	++	±
ADAMTS13	Normal/detectable	Normal/detectable
Tear drop cells Erythroblasts	0	+++
DIC	0	++
Treatment	Stop chemo	Start chemo

Conclusion

- **Significant understanding in anti-VEGF agents-associated TMA:**

  - The direct targeting of VEGF is sufficient to induce vascular injury and TMA; class effect

  - Role of VEGF in the structure and homeostasis of the slit diaphragm in humans

- **Anti-VEGF agents-associated TMA: may lead to intraglomerular TMA, kidney limited**

  sometimes only proteinuria/HT: need to be assessed !

- **Recovery of kidney function is more frequently seen (e.g., less severe course) after anti-VEGF interruption than in other chemo-induced TMA**

Conclusion

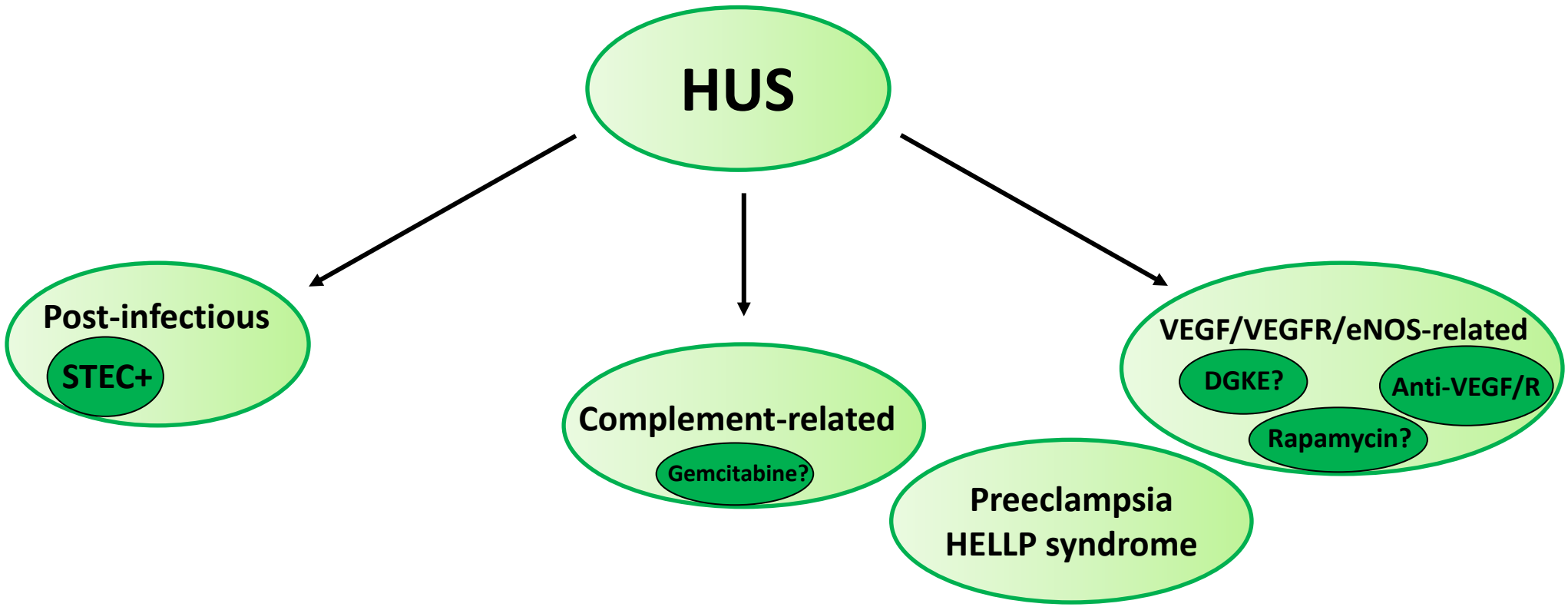
- **Complement blockage: a therapeutic perspective in gemcitabine-associated TMA; requires evaluation**

  Complement abnormalities need to be investigated in this context

- **Cancer-associated TMA and chemo-associated TMA need to be distinguished on the basis of clinical evaluation:**

- Disseminated intravascular coagulopathy	**Cancer-associated TMA**
- Circulating erythroblasts	
- Hypertension, renal insufficiency	**Chemo-associated TMA**

Antineoplastic agents-associated TMA: an extension of HUS spectrum?



Infections **Complement** **VEGF/VEGFR pathway**