Agensys

Changing Tomorrow Through
Cancer Research

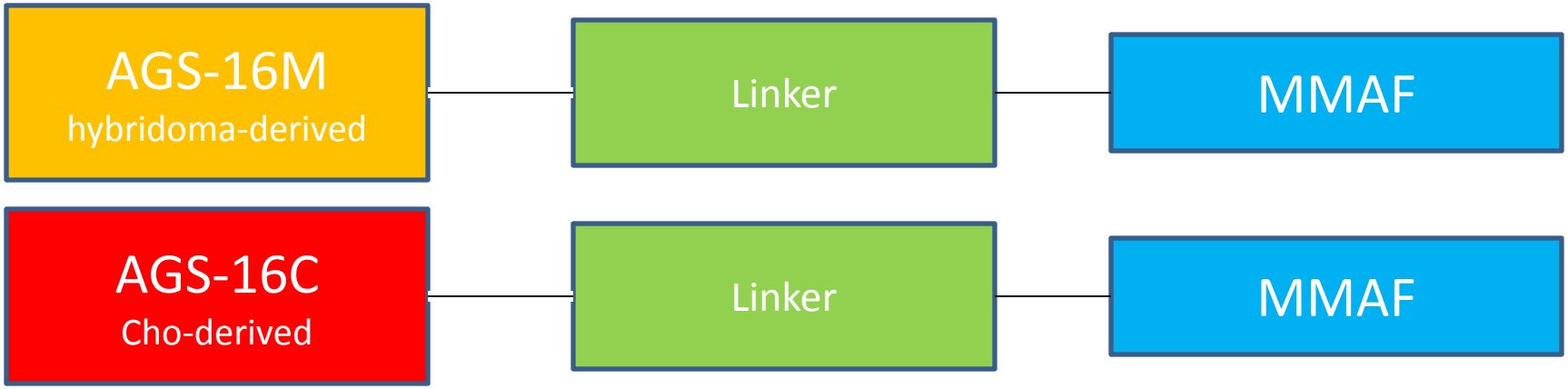
Anti-ENPP3 ADCs in Renal Cell Carcinoma

(AGS-16M8F/AGS-16C3F)

astellas

Leading Light for Life

AGS-16M8F and AGS-16C3F



Antibody:

- fully human IgG_{2k}
- targets ENPP3

  -in subset of tubules in kidney cortex
  activated basophils and mast cells,
  +/- fallopian tube, GI

  - 90% renal clear cell carcinoma (N =285);
  69% papillary renal cell carcinoma (N =39)
  27% hepatocellular carcinoma (N =320)

Linker:

- maleimidocaproyl linker
- non cleavable

monomethyl Auristatin F:

- Highly potent tubulin polymerization inhibitor
- hydrophilic; require antibody internalization

The Target: ENPP3

ectonucleotide pyrophosphatase/phosphodiesterase 3, also known as B10; NPP3; PDNP3; CD203c; PD-IBETA

- 875-aa type II cell surface phosphodiesterase (PDE)
- Member of the ENPP family

ENPP3 expression on cell membrane (by IHC):

- normal tissue
  - in subset of tubules in kidney cortex
  - in basophils and mast cells, upregulated upon activation
  - in fallopian tube, stomach, small intestine, and colon (variable expression)
- cancers
  - Kidney : 90% renal clear cell carcinoma (N =285); 69% papillary renal cell carcinoma (N =39)
  - Liver : 27% hepatocellular carcinoma (N =320)

ENPP3 functions in normal cells

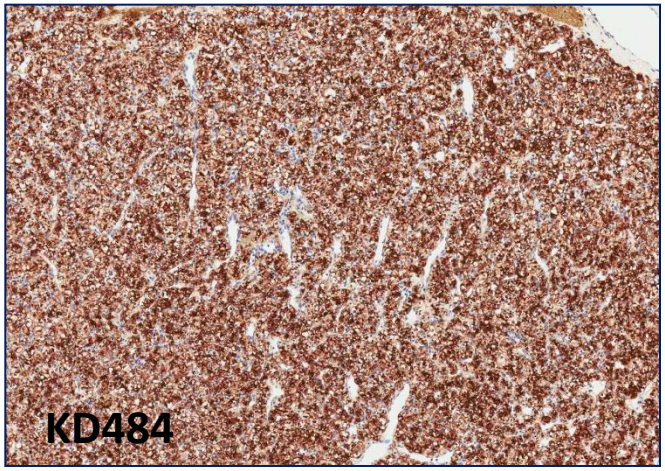
- in rat differentiation and invasion glial cells (mechanism unknown)
- ? renal cell response to inflammation (by increasing adenosine, which has anti-inflammatory proprieties)
- ? regulation of vascular and tubular functions (also by increasing adenosine)
- PPi regulation. Role in calcification?
- ? may interact with integrins
- ? role in purinergic signalling

ENPP3 function in cancer cells unknown.

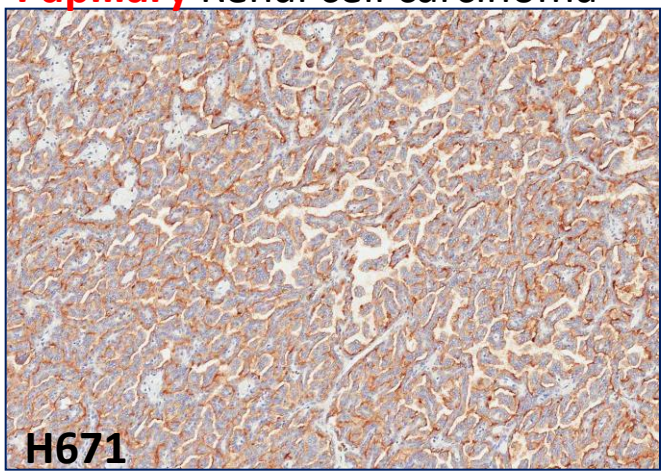
Long term (≈10 month) SD and clinical improvement in 1/ 7 subjects with advanced RCC treated in a Ph1 study of AGS-16M18, a fully human IgG1λ anti-ENPP3 naked Moab, at 1 or 3 mg/kg/ weekly

ENPP3 expression by IHC in cancer

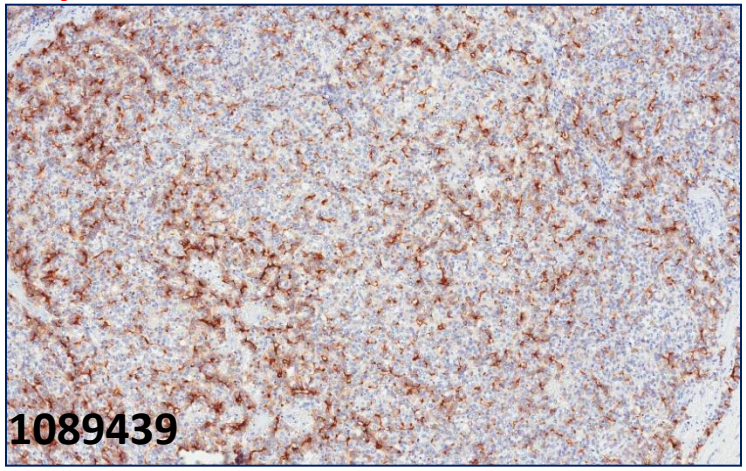
Clear cell Renal cell carcinoma



Papillary Renal cell carcinoma

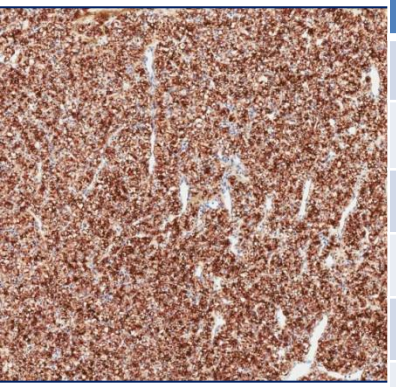


Hepatocellular carcinoma



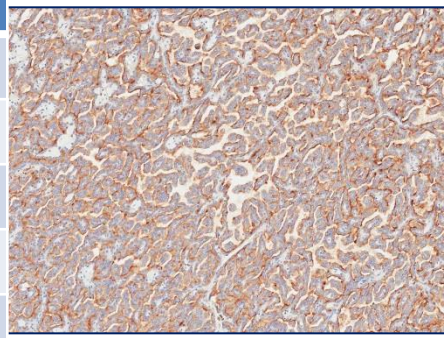
ENPP3 expression by IHC in RCC: tissue microarrays

clear cell carcinoma



Expression level	H-score	KIC1501	KD2085	KD2086	KD807	KD484	Total	Total %
High	**≥200-300**	28	73	78	24	36	**239**	**83.9**
Moderate	≥100-199	2	5	5	3	4	19	6.7
Low	≥15-99	0	1	4	2	2	9	3.2
Total Positive		30	79	87	29	42	**263**	**92.7**
Negative	0-≤15	1	6	8	0	3	18	6.3
Total		31	85	95	29	45	**285**	

papillary carcinoma



Expression level	H-score	KIC1501	KD2085	KD2086	KD807	KD484	Others*	Total	Total %
High	≥200-300	3	2	0	1	0	2	8	20.5
Moderate	≥100-199	0	0	2	2	0	7	11	28.2
Low	≥15-99	4	0	0	1	0	3	8	20.5
Total Positive		7	2	2	4	0	12	**27**	**69.2**
Negative	0-≤15	2	1	1	5	1	2	12	30.8
Total		9	3	3	8	1	14	**39**	

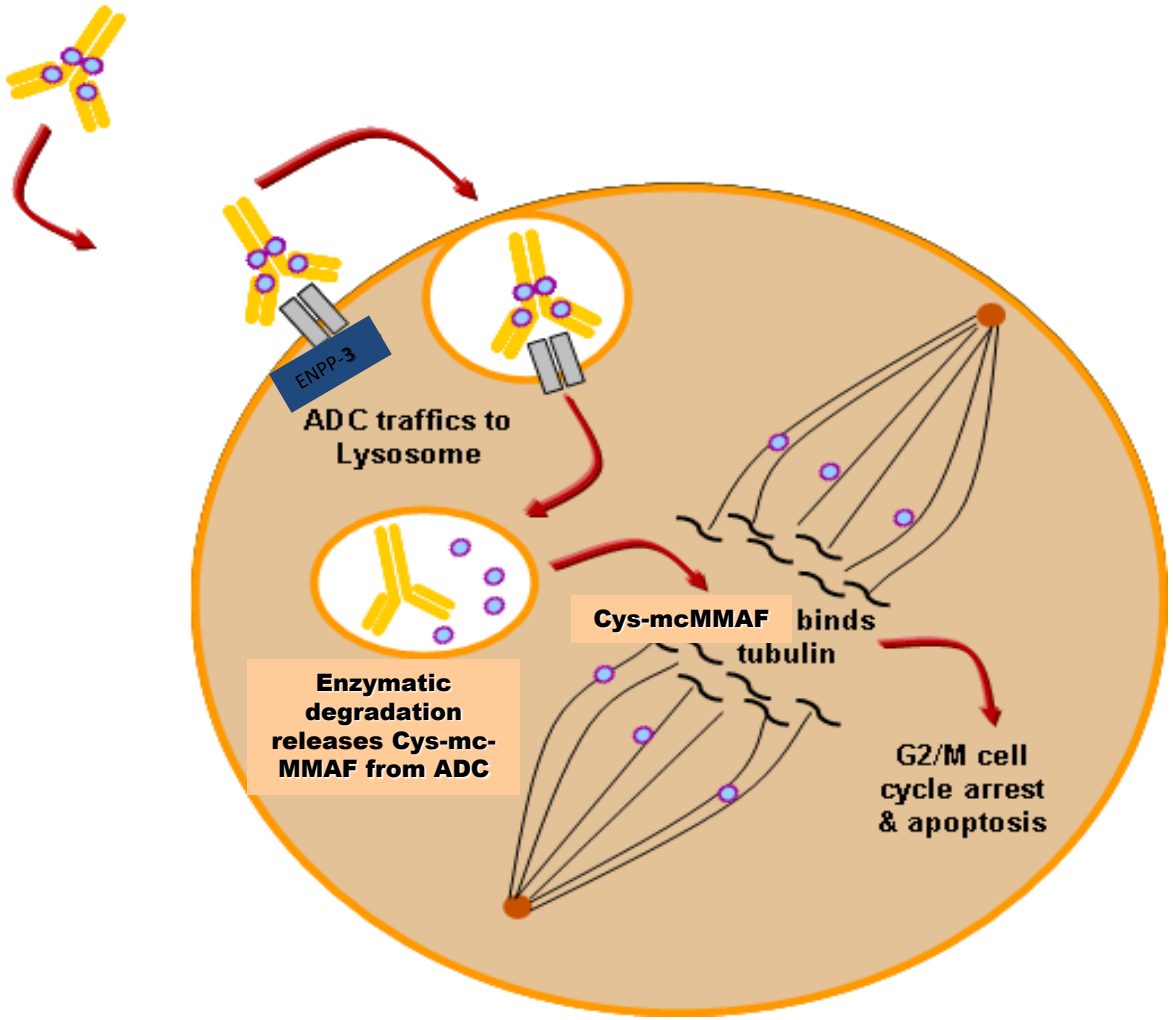
ENPP3 expression by IHC in RCC: samples from the 2009002 clinical study

ID	Pathology	From	ENPP3	H score
001	clear cell ca	bone met	pos	295
002	clear cell ca	abdom met	pos	295
003	clear cell ca	kidney lung	pos pos	275 290
004	clear cell ca	lung	pos	105
017	clear cell ca	GI	pos	286
007	clear cell ca	lung met	pos	135
008	clear cell ca	kidney	pos	140
009	clear cell ca	kidney	pos	280
012	clear cell ca	kidney	pos	195
013	clear cell ca (with sarcomatoid ca foci)	kidney	pos	277
010	clear cell ca (eosinophilic variant)	kidney	low pos	<0.1
022	clear cell ca (with eosinophilic variant)	kidney	lo pos	0.6
015	papillary ca	kidney	neg	0
006	papillary ca	node met	neg	0
018	papillary ca (with clear cell ca foci)	kidney	low pos	20
025	unclassified (acinar and papillary)		pos	258

AGS-16M8F: Mechanism of Action

AGS-16M8F

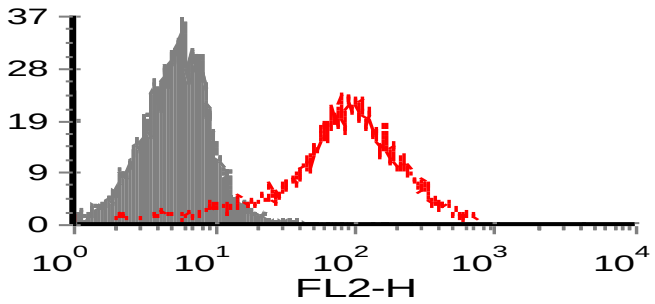
- binds ENPP3
- ENPP3/AGS-16M8F complex is internalized
- traffics to the lysosomes where the ADC is completely degraded, releasing Cys-mc-MMAF
- Cys-mc-MMAF is a potent hydrophilic anti-tubulin agent



- **binds ENPP3**

AGS-16M8F binds to cell surface ENPP3 (and mediates cytotoxicity *in vitro)*

A



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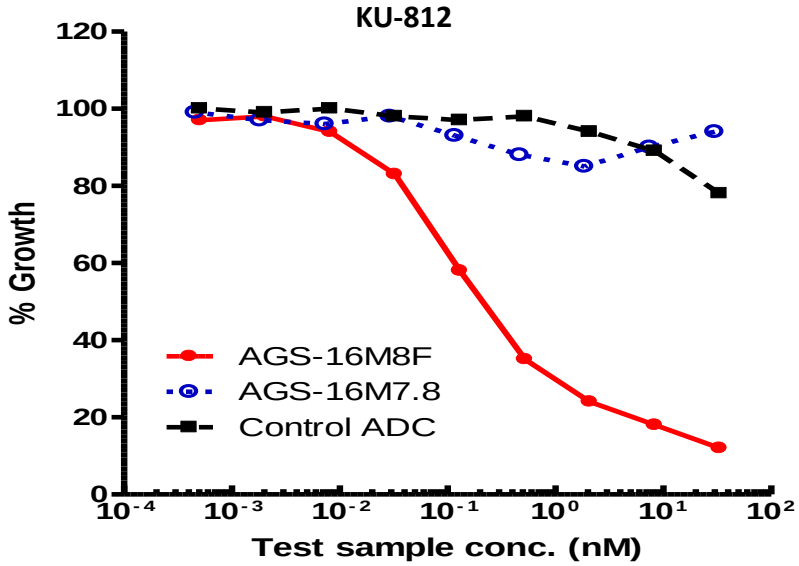
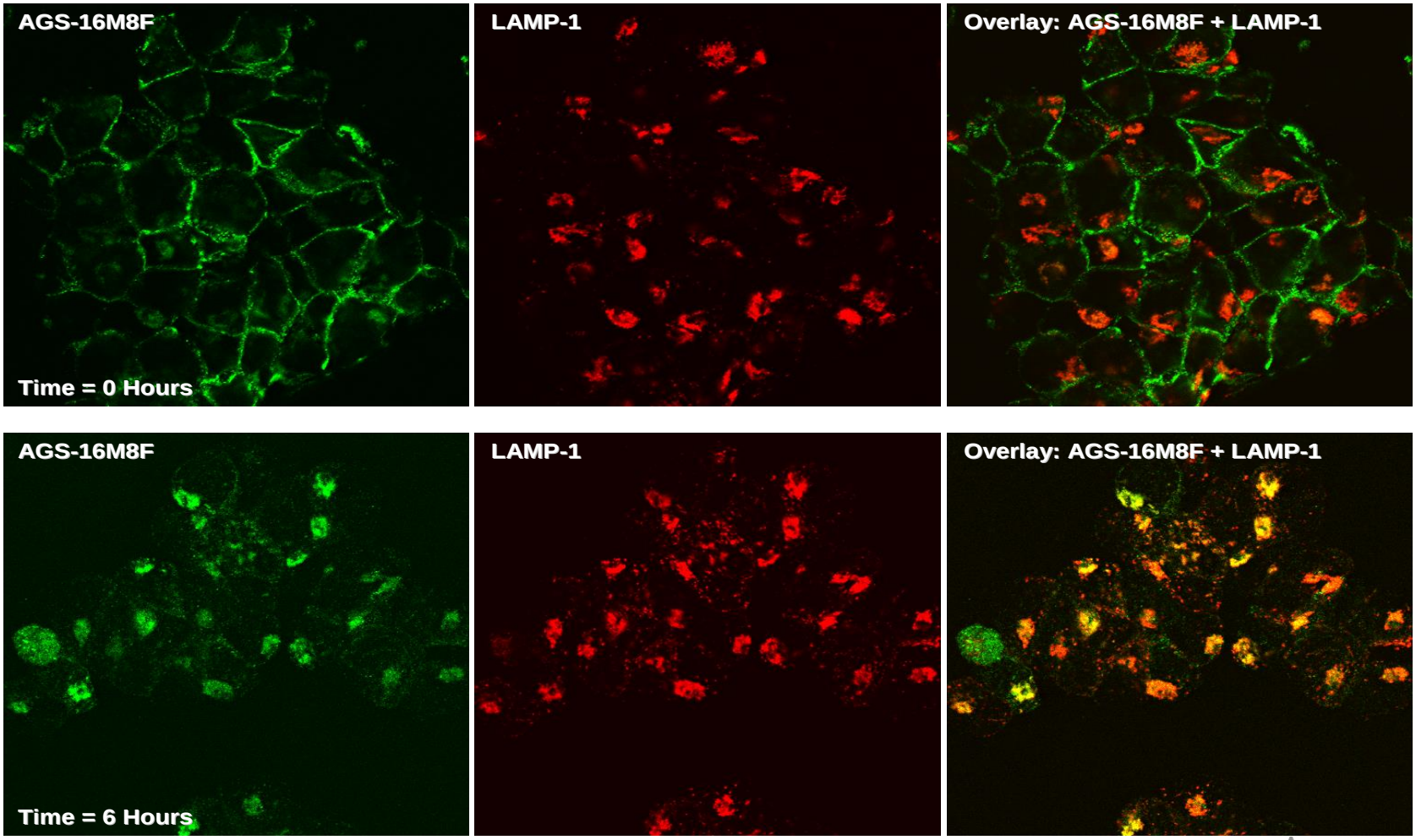


- AGS-16M8F binds to cell surface ENPP3 and mediates cytotoxicity on KU-812 cells (IC_{50} ~ 0.2 nM)
- Native MAb (AGS-16M7.8) is not cytotoxic
- Control ADC conjugated with mc-MMAF is not cytotoxic

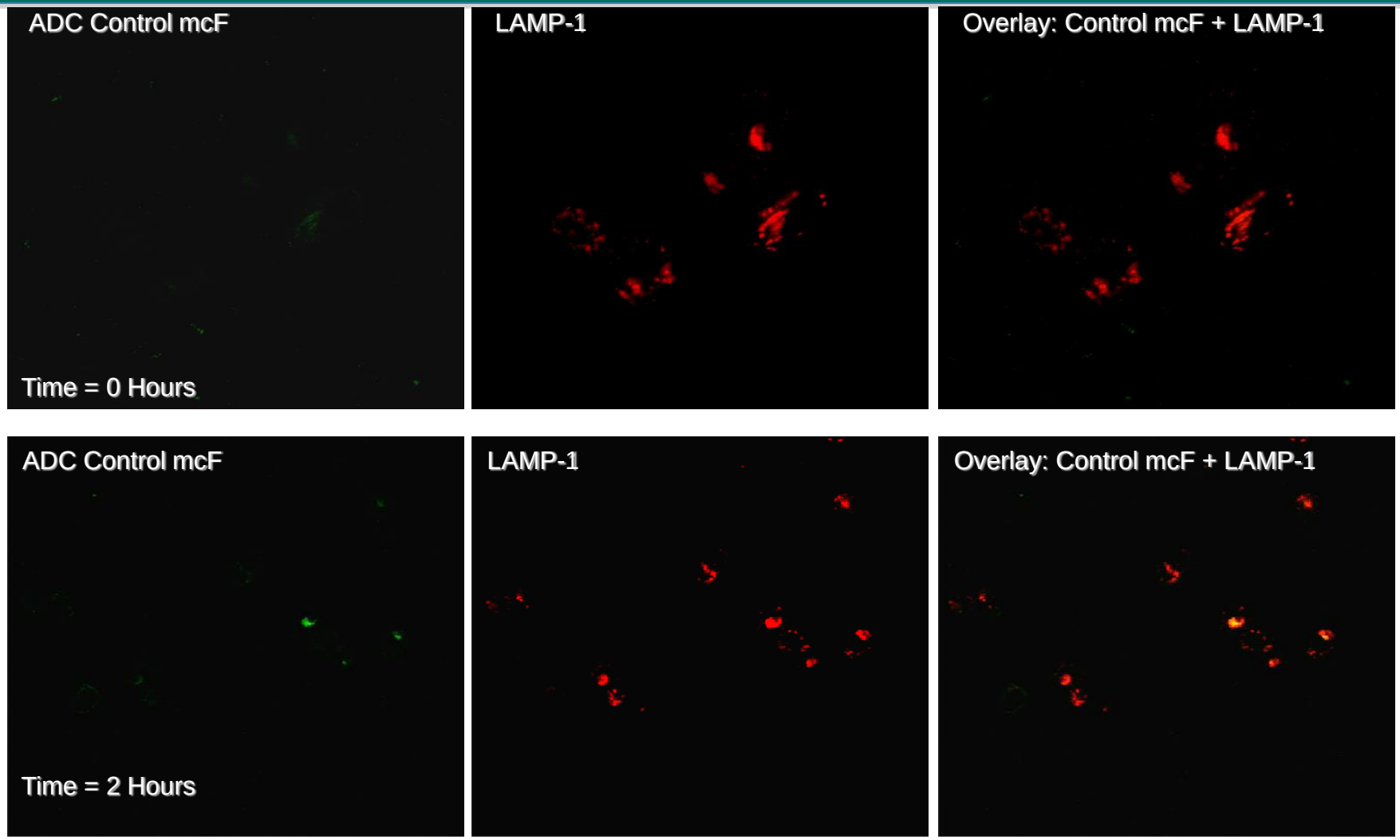
- **ENPP3/AGS-16M8F complex is internalized**
- **traffics to the lysosomes**

AGS-16M8F mediates internalization of ENPP3 and trafficking to lysosomes in KU- 812 cells *in vitro*



10µg/mL AGS-16M8F Green- AGS-16M8F Red- LAMP-1

(control mcMMAF Does Not Bind or Traffic to Lysosomes In Vitro)



Green- H3-12abc-mcF Red- LAMP-1

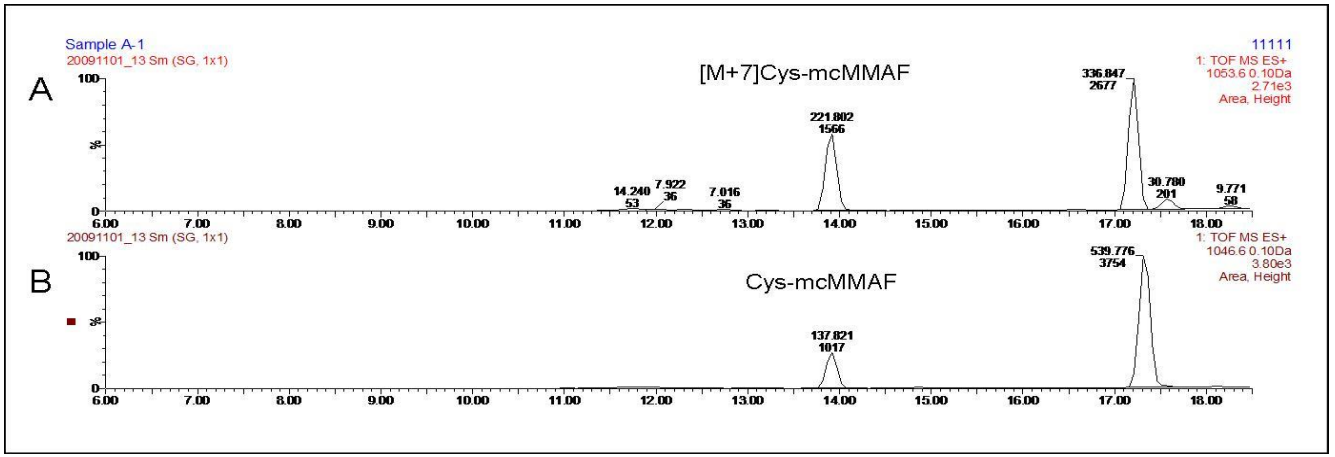
.....where the ADC is completely degraded, releasing Cys-mc-MMAF

Mass spec analysis of metabolites detected from UG-K3 tumors treated with AGS-16M8F

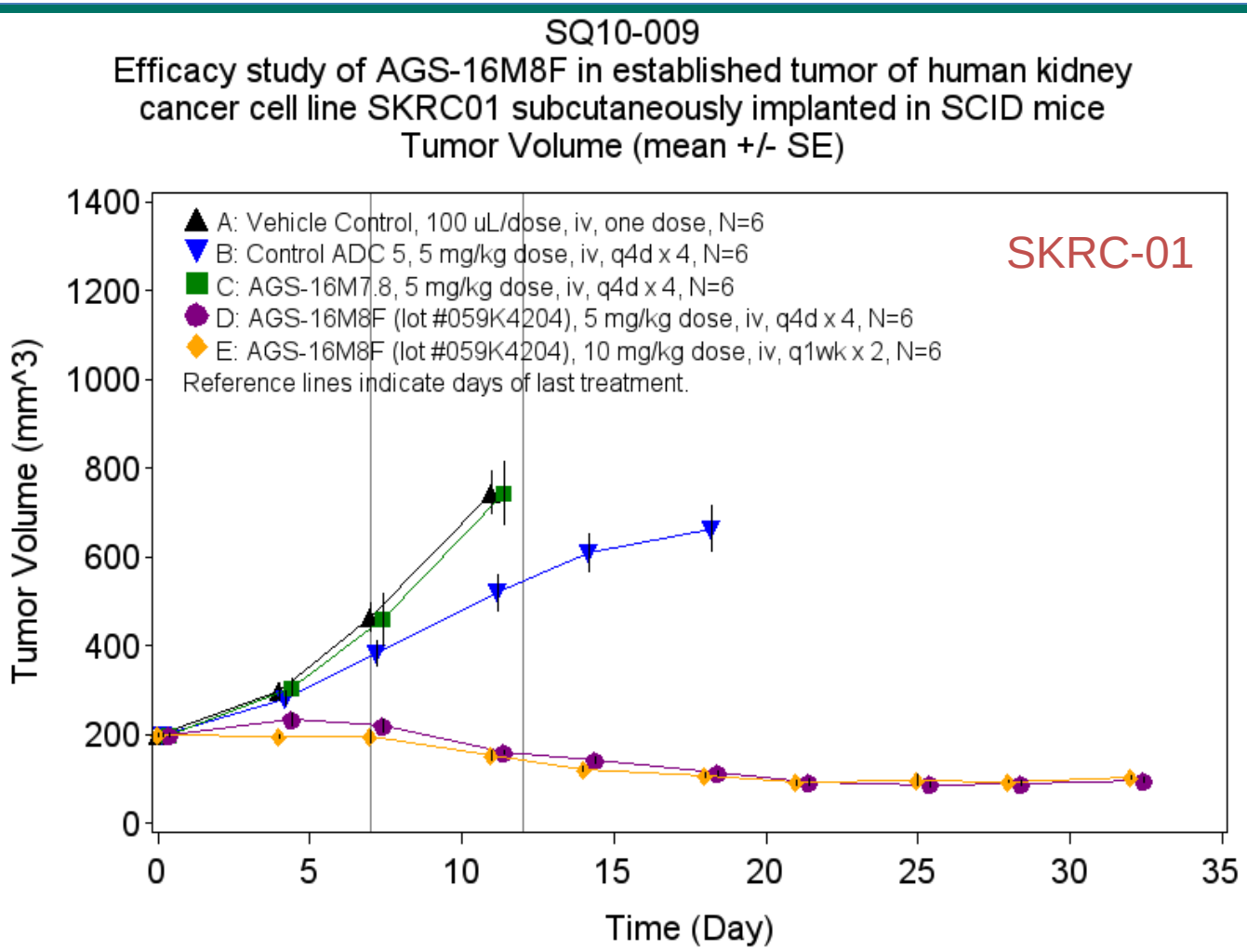
Cys-mc-MMAF was the only drug related metabolite detected by Mass Spec

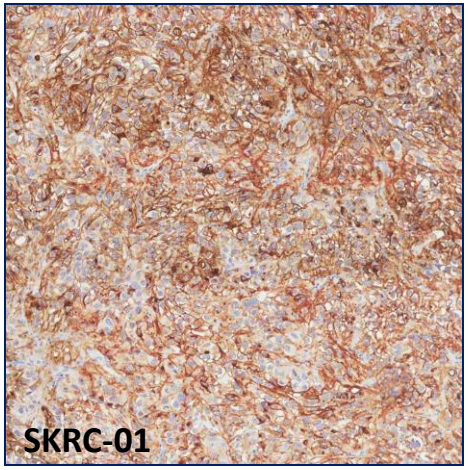
	Cys-mc-MMAF	
Time (days)	nM	pmol/g tumor
1 (Control)	ND*	ND*
1	16.1 ± 0.7	47
3	14.8 ± 4.9	35
5	5.0 ± 1.4	19

*ND: not detected

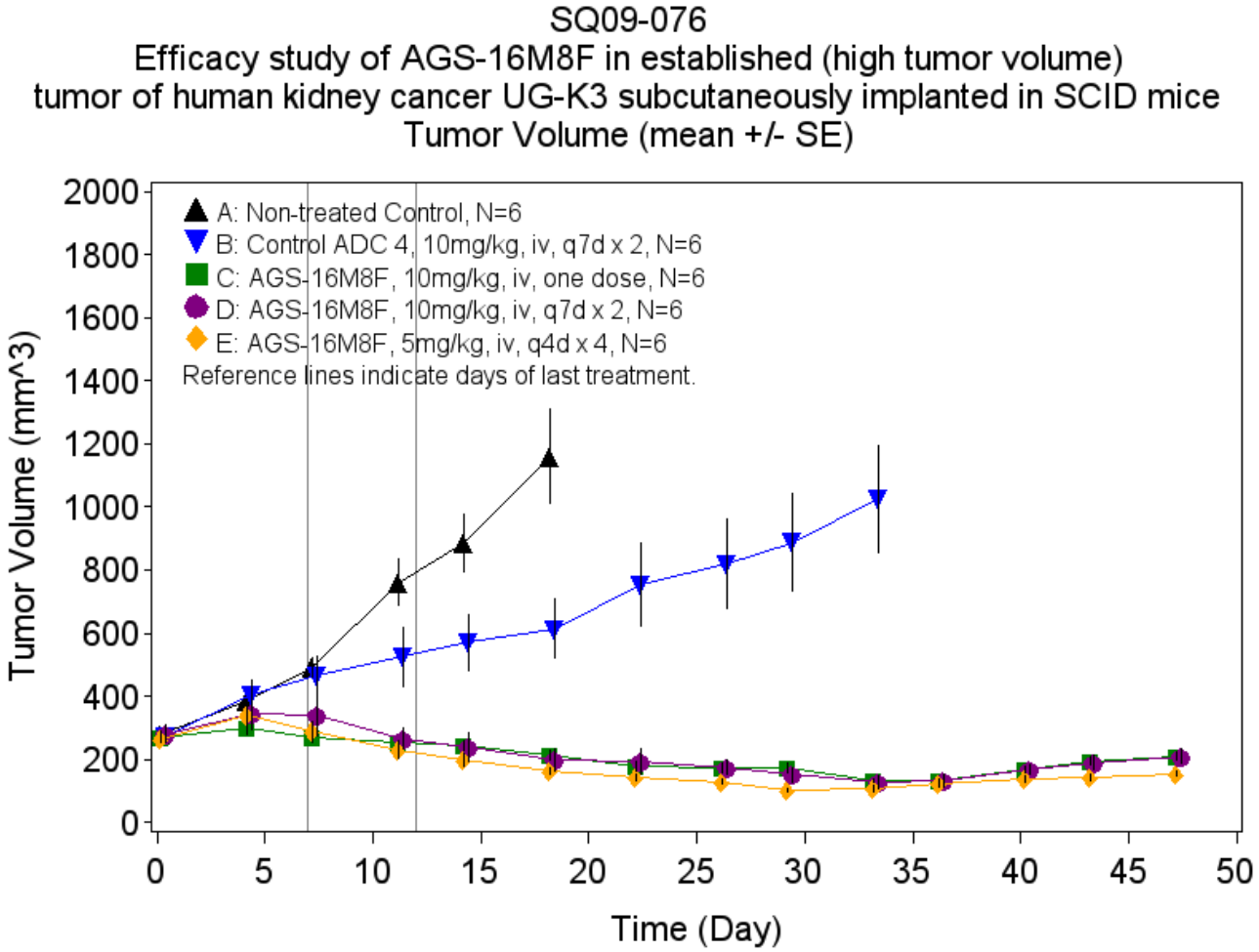


AGS-16M8F inhibits the growth of established renal clear cell carcinoma SQ xenograft (SKRC-01)

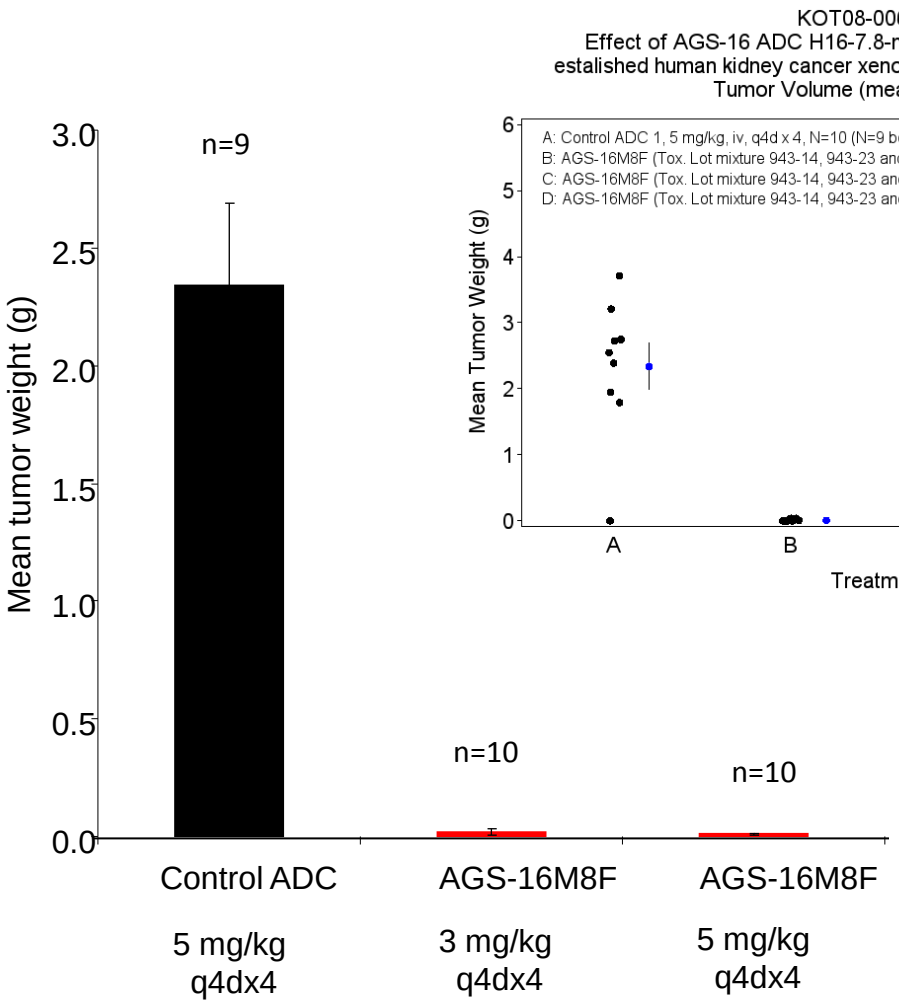


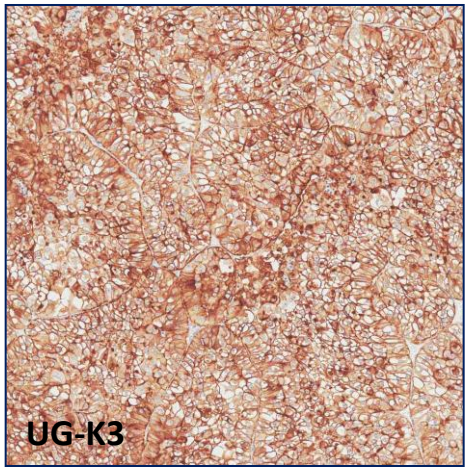


AGS-16M8F regresses large established SQ kidney cancer xenografts (UG-K3)



AGS-16M8F treatment regressed established orthotopic renal clear cell carcinoma xenograft (UG-K3)



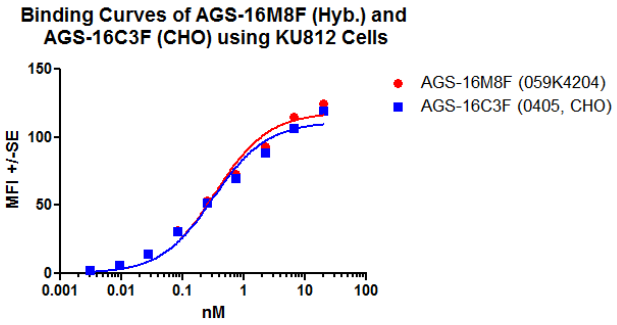


- UG-K3 is a patient derived renal clear cell carcinoma xenograft
- Tumors were implanted orthotopically and established for 7 days
- IHC analysis demonstrates moderate-strong expression of ENPP3 in UG-K3

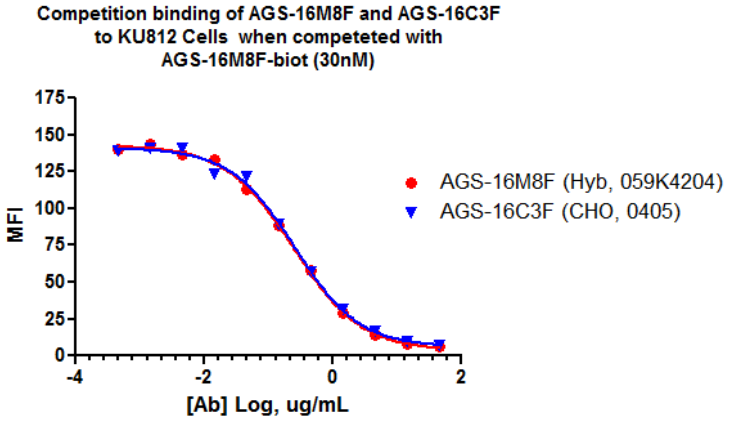
AGS-16M8F and AGS-16C3F have similar biological activities

Objective	Study Title	Key Results	Study/Report No.
Binding	Comparison Study of Hybridoma Derived AGS-16M8F (Lot 041M7253) and CHO Derived AGS-16C3F (Lot 041M4215) using AGS16 Antigen (ENPP3) Binding ELISA	Demonstrated that the relative **potency for binding to ENPP-3** of AGS-16C3F to be **95.2%** of AGS-16M8F in an ELISA format indicating similar affinity	RD11-002
Binding	AGS-16C3F and AGS-16M8F Bind to Human AGS-16 Antigen (ENPP3) Expressed on the Surface of KU812 Cells	Results showed that both AGS-16C3F and AGS-16M8F **bind specifically and with similar affinity** to the human ENPP3 antigen expressed on the surface of KU812 cells in vitro.	RD11-003
Binding	AGS-16C3F and AGS-16M8F Compete for the Same Epitope of the AGS-16 Antigen (ENPP3)	The results from this experiment suggest that both antibodies **bind the same epitope within ENPP3** and with the same affinity within the experimental error of the assay	RD11-004
Cytotoxicity	Determination of AGS-16C3F and AGS-16M8F Cytotoxicities on KU812 Cells	This experiment demonstrate that treatment of AGS16-expressing cells, KU812 cells, with AGS-16C3F induces potent **cytotoxic activity similar** to that of AGS-16M8F	RD11-005
In vivo efficacy	Efficacy Study of AGS-16M8F and AGS-16C3F in a Subcutaneously Established Xenograft Model of Human Renal Cancer UG-K3 in SCID Mice	This experiment demonstrated potent antitumor activity for both AGS-16M8F and AGS-16C3F in a dose dependent manner. **no**There were **no statistically significant differences observed** between the two ADC products at any of the three dose levels tested. Furthermore, visual inspection of the results indicates similar efficacies, within the experimental error of the method, for AGS-16M8F and AGS-16C3F at any of the three doses tested.	RD11-001

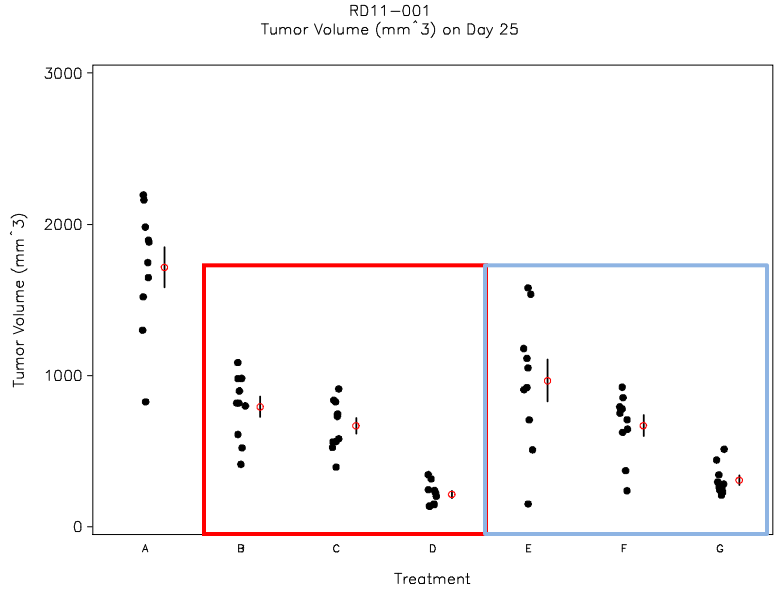
AGS-16M8F and AGS-16C3F have similar biological activities



	AGS-16M8F (059K4204)	AGS-16C3F (0405, CHO)
One site binding (hyperbola)		
Best-fit values		
Bmax	118.1	111.1
Kd	0.3372	0.3154



	AGS-16M8F (Hyb, 059K4204)	AGS-16C3F (CHO, 0405)
IC50 (ug/mL)	0.25	0.26



A.- H3-1.4.1.2-mcF , 0.5 mg/Kg, twice weekly, 4 doses, i.v., N=10

B.- AGS-16M8F , **0.125** mg/Kg, twice weekly, 4 doses, i.v., N=10

C.- AGS-16M8F , **0.25** mg/Kg, twice weekly, 4 doses, i.v., N=10

D.- AGS-16M8F , **0.5** mg/Kg, twice weekly, 4 doses, i.v., N=10

E.- AGS-16C3F , **0.125** mg/Kg, twice weekly, 4 doses, i.v., N=10

F.- AGS-16C3F , **0.25** mg/Kg, twice weekly, 4 doses, i.v., N=10

G.- AGS-16C3F , **0.5** mg/Kg, twice weekly, 4 doses, i.v., N=10

non-clinical toxicology:

in vitro:

•ADCs do not cause mast cell degranulation

in cynomolgus monkeys:

- AGS16M cross-reactive with cynomolgus monkey ortholog
- treatment with AGS16M8F or naked antibody well tolerated when given at a dose up to 6 mg/kg weekly for 4 weeks
- no test article related target organs were identified in non-human primates.

  in particular:

  -no corneal toxicity was noted during ophthalmological exams (biomicroscopy)

  -minimal change was noted in platelet count (22-42%) compared to concurrent control values for males only at the 6 mg/kg dose. All platelet values within reference range for primates; reversible after last dose and therefore not considered adverse no corresponding clinical findings or microscopic changes

Clinical Development

- Study 2009002, ph1 study of AGS-16M8F
  Closed. MTD not reached at 4.8 mg/kg

- Study AGS-16C3F-12-2
  ongoing
  -Bridging/Dose Finding Phase started at the dose found safe for hybridoma product (4.8 mg/Kg)
  -Planned: Dose expansions in cancers with clear cell histology
  (in enpp3+ cancers of papillary histology)

- Further expansion of combination therapy considered
  (preclinical evidence of synergy)

Study Overviews

- Phase 1 trials in patients with advanced metastatic renal cell carcinoma:
  - Hybridoma: no restriction regarding prior therapies or ENPP3 status
  - CHO: restricted to VGF inhibitor failures in clear cell histology.
    restricted to ENPP3 positivity in other histologies
- ADCs given IV q3w
- MTD evaluation based on adverse events occurring in the first 3 weeks of therapy
- therapy continued until toxicity, disease progression or otherwise determined by the investigator
- disease evaluation performed q12w (hybridoma) or q8w (CHO)

AGS-16M8F: Phase 1 in advanced RCC. Subject Characteristics

Data Item	Data
Gender	19 Male, 7 Female
Age	47 - 80 years (median 65)
BMI >30	13
Histology	19 clear cell ca (and variant)
	3 papillary ca
	2 unclassified ca
Prior DiseaseDuration	0.6 to 30 years (median 6.6 years)
Prior Tx History	Chemotherapy: 17 subjects
	Radiotherapy: 5 subjects
# of AGS-16M8F dose	1-15 dose/patient (median 4)

Study AGS-16C3F-12-2 : Subject Characteristics (as 4/30/2013)

Data Item	Data
Gender	9 Males, 2 Females
Age	47-53 (median 57) years
BMI >30	5
Histology	8 clear cell ca
	1 papillary ca
	2 others
Prior Disease Duration	1.6- 17 (median 6.2) years

AGS-16M8F: Phase 1 in advanced RCC. Subjects Disposition.

Cohort/Dose	Enrolled	Outcome
1 (0.6 mg/kg)	6	1 DLT
2 (1.2 mg/kg)	3	
3 (1.8 mg/kg)	3	
4 (2.7 mg/kg)	3	
5 (3.6 mg/kg)	3	
6 (4.8 mg/kg)	8	
7 (6.0 mg/kg)	0	
8 (7.0 mg/kg)	0	
9 (8.0 mg/kg)	0	
Total	**26**	

Study AGS-16C3F-12-2 (as 4/30/2013)

Cohort/Dose	Enrolled	Outcome
1 (4.8 mg/kg)	2	2 DLT
0 (3.6 mg/kg)	6	1 DLT 4 off study after ≥2nd dose 1 ongoing
-1 (2.7 mg/kg)	3	3 ongoing

AGS-16M8F: Phase 1 in advanced RCC Summary of Results

- Dose Limiting Toxicities (DLTs)
  - Cohort 1: Pulmonary embolism and angina
- MTD criteria not reached at 4.8 mg/kg
- PK (preliminary data):

  - long half-life for ADC (individual range- 3.07 – 11.88 days; n=24) and TAb (individual range- 4.56 – 16.5 days; n=24).

  -serum ADC concentrations decreased multi-exponentially following the end of infusion. Both serum AUC and Cmax increased in an approximately dose-proportional manner
- Best response:
  - Cohort 1 (#002, 003, 004): Prolonged Stable Disease (disease control≈ 6, 6 and >10 months)
  - Cohort 4 (#013): Prolonged Partial Response (disease control≈ 20 months)

Study AGS-16C3F-12-2: Preliminary Results

- Dose Limiting Toxicities (DLTs)
  - Cohort 1: #1-0001:ocular toxicity
    #1-0002:Transient posterior encephalitis (prior use of Avastin)
  - Cohort 0: #6-0007: thrombocytopenia (+ ocular toxicity)
- 3.6 mg/kg met protocol criteria for MTD;
  still searching for a dose with long term tolerability

AGS-16M8F: Phase 1 in advanced RCC Relevant Treatment Emergent AEs

Adverse Event	Relevance	Grade	Incident rate	Dose Dependent?
Pulmonary embolism	DLT in cohort 1	4	<5%	No
Visual disturbance (allucination)	unconfirmed DLT cohort 6	2	<5%	No
Constipation	Typical tubulin inhibitor side effect	1,2	27%	No
Nausea		1,2	23%	No
Fatigue		1 to 3	50 %	No
Dyspnea Cough		1,2 1,2	23% 15%	No
Thrombocytopenia	"new ADC-related complications"	3	30%	No
Eye dryness/itching Blurred vision		1,2	15%	Yes
dizziness Infusion related rash/itching	ADC vs target related	1-3	19% 15%	?
Bleeding (RCC-related GI bleeding*, nose bleeding)		1 to 3	≈10%	No

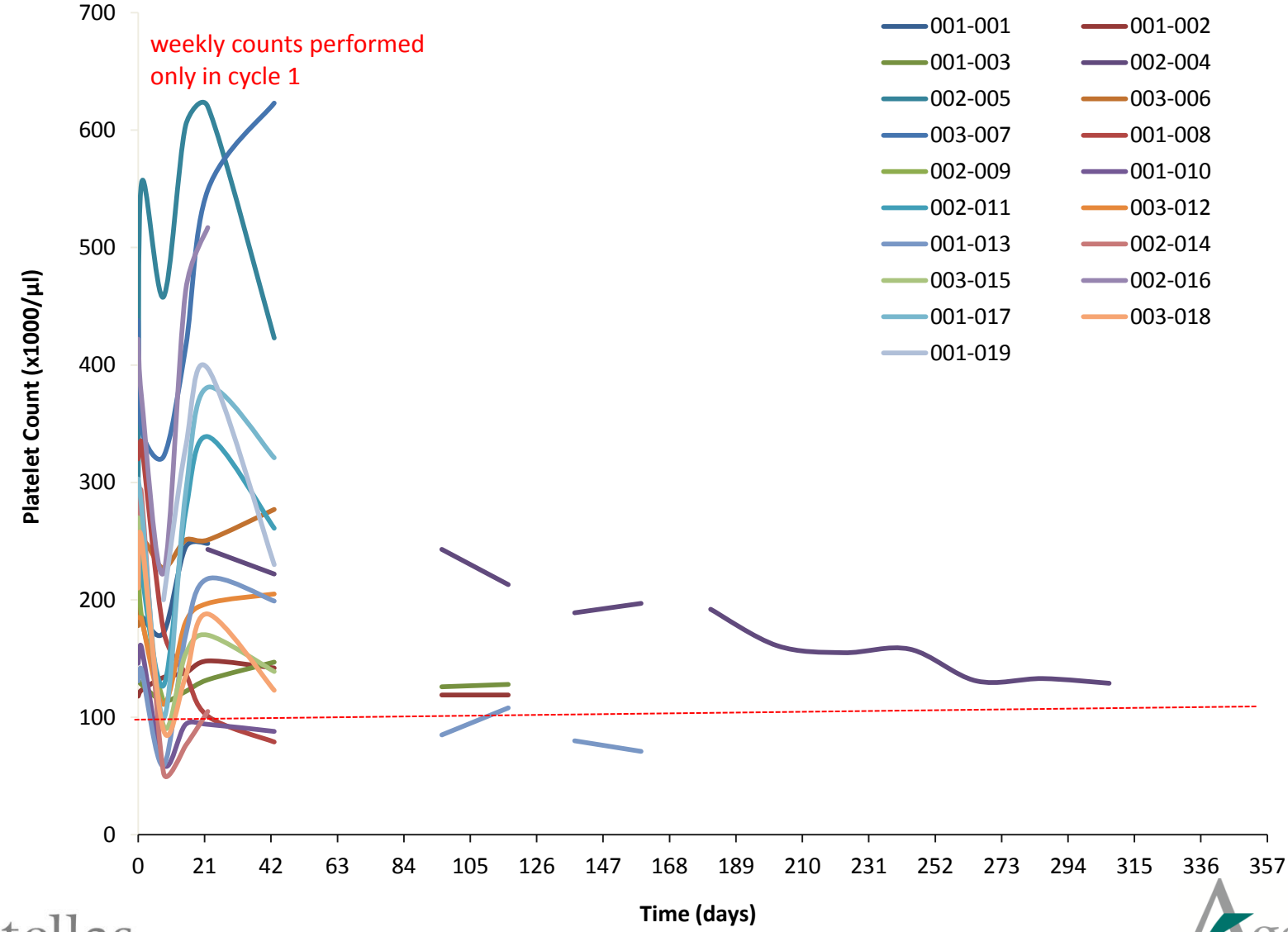
* occurred while normal platelets count

Isolated Thrombocytopenia

- Two “types” of thrombocytopenia observed in the hybridoma study:
  - Acute and transient platelet drop (nadir at Day 7, recovery at Day 21) in 70% patients receiving ≥1.2 mg/kg
    - Not clearly dose related
  - Slow and progressive late platelet decrease (after ≥day 42) in ≈50% patients treated for six or more weeks
    - Not dose related
  - no major episodes of bleeding in thrombocytopenia

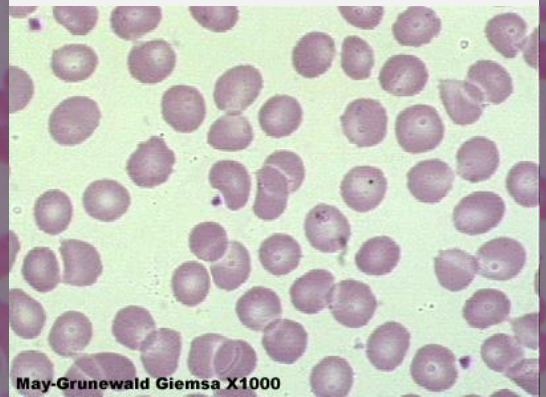
- **Isolated Thrombocytopenia is also reported after treatment with other tubulin-inhibitor based ADCs and naked antibodies**
- Etiology is unknown. Hypotheses includes:
  - defects in MK endomitosis and/or demarcation caused by tubulin inhibition
  - antibody-mediated increased platelet clearance (ITP or heparin-like)
  - direct effect on platelet tubulin altering margination and/or accelerating clearance
  - effect on endothelium causing platelet adhesion/ margination

Platelet Response after AGS-16M8F Treatment



day>100
016

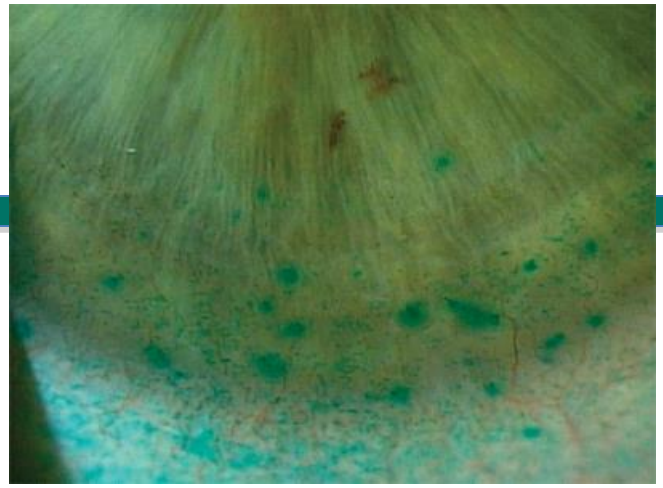
wiscott aldrich



Thrombocytopenia-CHO study preliminary results

- 9 of 10 evaluable subjects had a platelet drop at day 7 reaching 13 – 155 (median 78) $x10^9/L$
- complete recovery at day 21
- minimal variation in MPV during the first cycle (always <11 fl)

Isolated Corneal toxicity



- **Dry eye" and blurred vision due to corneal toxicity reported in phase 1 studies of other ADCs conjugated with tubulin inhibitors, including MMAF, MMAE, DM4 and DM1:** ophthalmology exam in selected patients revealed corneal damage consistent with punctated epithelial keropathy. in others the corneal damage was defined as aspecific.

- possible pathogenesis include damage in lacrimal glands with decreased tear production and/or cytotoxicity from ADC in tears vs blood

Ocular toxicity characteristics. Hybridoma study

#	dose		grade	start	duration
10	(1.8 mg/kg)	dry eyes	? ?	post dose 2 post dose 3	? ?
13	(2.7 mg/kg)	dry eyes	1	day 18	NR
17	(3.6 mg/kg)	dry eyes	1	day 24	2 days
19	(4.8 mg/kg)	dry eyes	1	day 35	NR
22	(4.8 mg/kg)	dye eyes/ blurred vision	2 1	day 23 day 43	19 days NR
24	(4.8 mg/kg)	blurred vision dry eyes	2 1	day 24 day 43	NR NR
25	(4.8 mg/kg)	blurred vision	2	day 22	NR
26	(4.8 mg/kg)	blurred vision conj hemorrhages	2-3	day 10	NR

Hybridoma study: 8 subjects developed dry eyes with or without blurred vision mostly after the second AGS-16M8F dose. Symptoms were transient but often recurred after following doses. Ophthalmology exams in three subjects; in 2: aspecific corneal findings; in 1 : corneal and conjuntival abnormalties (connular keratits , hemorrhagic conjuntivitis) and refraction disorders

AGS-16C3F: Isolated corneal toxicity preliminary results

- ophthalmology exam mandated at baseline and every 2 doses +PRN

- post therapy exam so far available in 7 subjects (1 cohort 1, 6 cohort 0)

✓ *corneal toxicity in 7/7 subjects after 1 or 2 doses*
(reported as presence of microcysts, punctuated staining, deposits or erosions)

✓ *visual acuity decreased (at least one eye) to:*
- *≥20/150 in 2 subjects*
- *>20/40 in 3 subjects*
- *lesser vision loss in 2 subjects*

✓ *tear production impaired in 2/2 subjects who had the Schirmer test*

- follow up exams in the 2 subjects with ≥20/150 decrease: partial recovery after 2 to 6 weeks (FU ongoing)

- discrepancies between corneal finding and visual complains : it might depend on location of the corneal damage (lesions beginning in periphery and migrating to the center?)

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Anti-cancer activity

Best Response

26 subjects in the hybridoma study: 8 not evaluated (early off study for toxicity, etc)
9 PD
8 SD
1 PR

11 subjects in the CHO study: 7 not evaluated (early off study for toxicity)
1 SD
3 too early

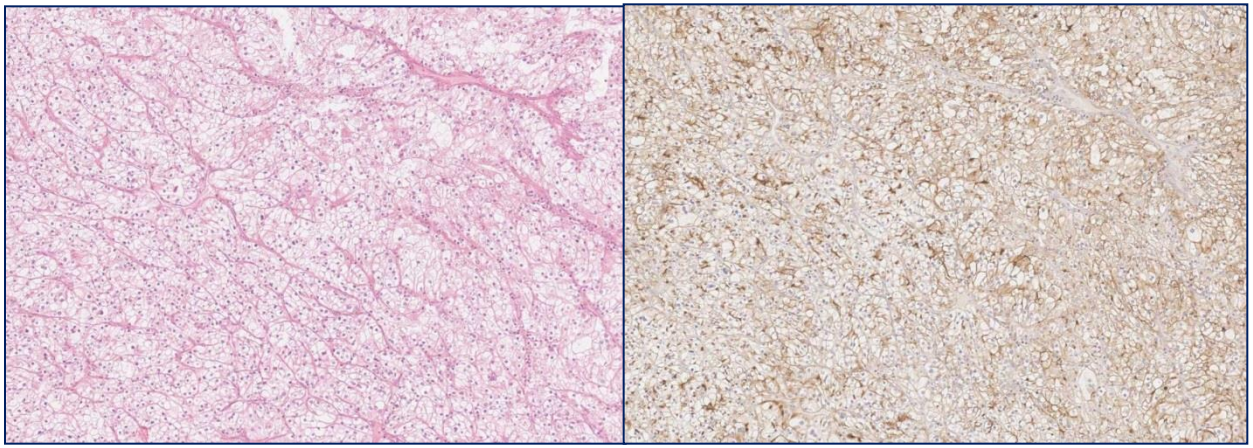
hybridoma study: duration of disease control in 15 subjects with at least a disease evaluation at ≈12 w.

	dose mg/kg	max response	duration (days from 1st dose)	cause off-study
002	0.6	SD (3.9%↑)	183	PD
003	0.6	SD (9.7% ↓)	182	PD
004	0.6	SD (3.4% ↓)	336	PD
005	0.6	PD	77	PD
007	1.2	PD	84	PD
008	1.2	PD	92	PD
009	1.2	PD	91	PD
010	1.8	PD	92	PD
011	1.8	SD (4.5% ↑)	139	PD
012	1.8	PD	84	PD
013	2.7	PR (50.3% ↓)	581	PD
015	2.7	SD (16.1% ↑)	98	inv decision
017	3.6	SD (23.1% ↓)	88	inv decision
022	4.8	SD (16.7% ↑)	92	inv decision?
024	4.8	SD (5.4% ↓)	170	AE (gr2)

Pathology, ENPP3 Expression and Tumor Response

ID	Pathology	From	ENPP3	H score	Response
001	clear cell ca	bone met	pos	295	not eval
002	clear cell ca	abdom met	pos	295	SD →PD@22w
003	clear cell ca	kidney lung (post)	pos pos	275 290	SD →PD@36w
004	clear cell ca	lung	pos	105	long term SD (10m)
006	papillary ca	node met	neg	0	PD@6w
007	squamous cell ca (?) clear cell ca	lung met lung met	pos NE	135 NE	SD →PD@16w
008	clear cell ca	kidney	pos	140	PD@10w
009	clear cell	kidney	pos	280	PD
010	clear cell ca (eosinophilic variant)	kidney	low pos	<0.1%	PD@8w
012	clear cell ca	kidney	pos	195	PD@9w
013	clear cell ca (with sarcomatoid ca foci)	kidney	pos	277	long term PR (>1y)
015	papillary ca	kidney	neg	0	not eval
017	clear cell	GI	pos	286	SD 6W
018	papillary ca (with clear cell ca foci)	kidney	low pos	20	PD@9w
022	clear cell ca (with eosinophilic variant)	kidney	lo pos	0.6	PD @12 w
025	unclassified (acinar and papillary)		pos	258	

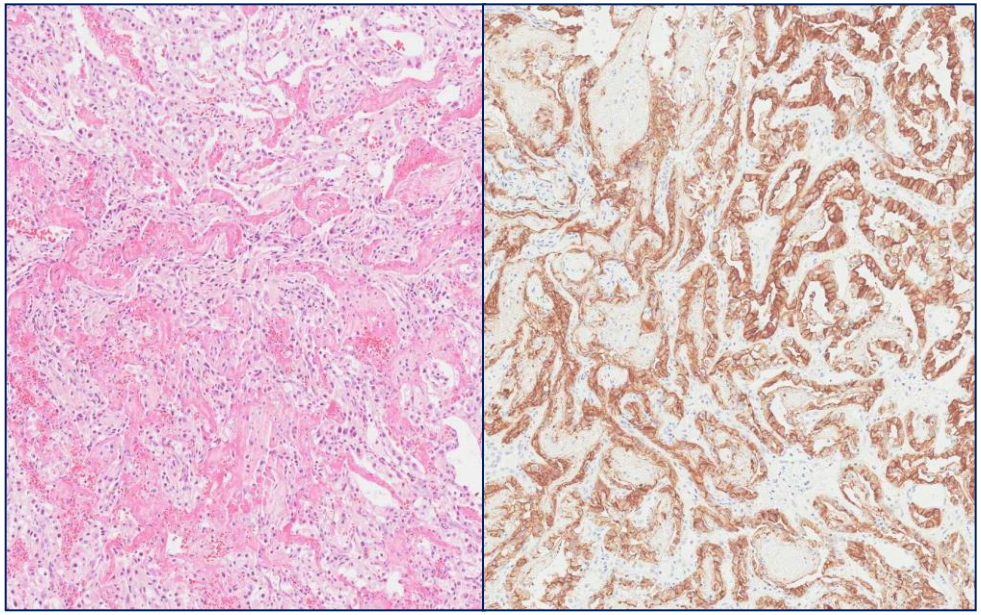
ENPP3 status after therapy: #001-003



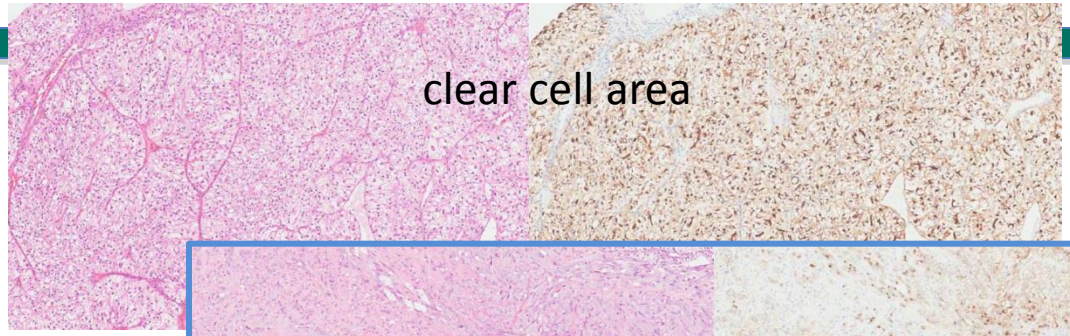
archived kidney cancer

0.6 mg/kg q3w
First dose 10/4/2010

biopsy lung
metastases post therapy
March 31, 2011

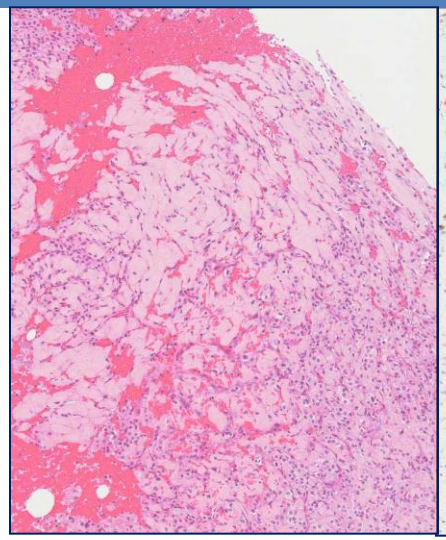


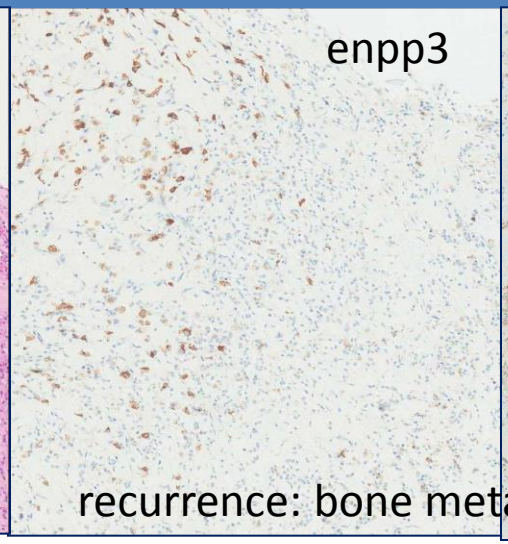
ENPP3 status after therapy: #001-013

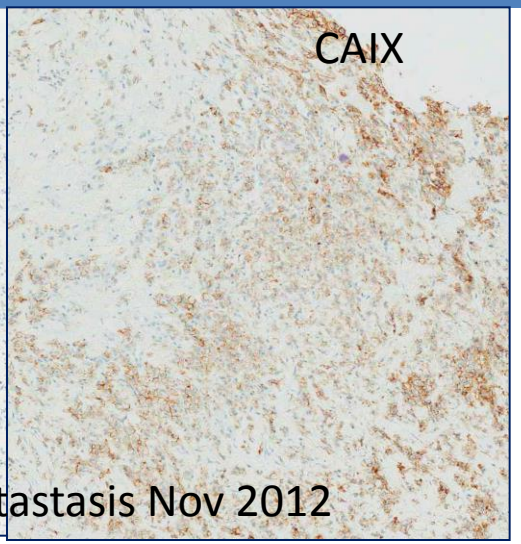


archived kidney cancer
(excised Sept 2002)

treated at 2.7 mg/kg q3w from April to Sept 2011 (got PR), then q4 w to July 2012, then q5 week onwards.



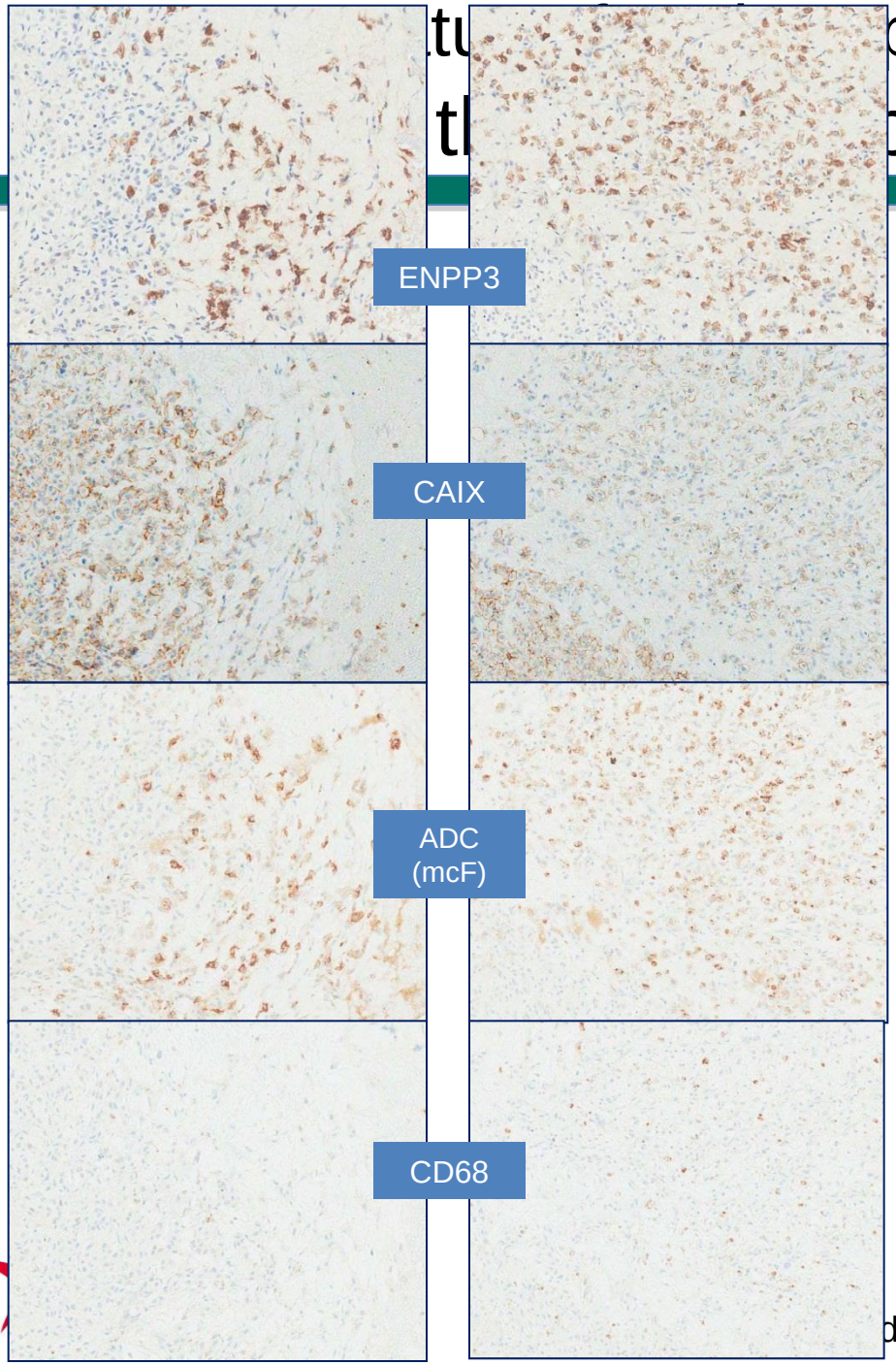




recurrence: bone metastasis Nov 2012

ENPP3 status after therapy: #001-013.
What are the ENPP3 positive cells?

tu by: #001-013.
t positive cells?



- staining for ENPP3, ADC and macrophages have a similar distribution (ENPP3 + cells maybe dying or phagocytated by macrophages)
- CAIX positive cells look fine, minimal drug presence or CD68 infiltration

Hypothesis:
ADC reached ENPP3 positive cancer cells causing cell death and macrophage infiltration. ENPP3 negative cancer cells are unaffected by therapy and their growth caused the progression.

Agensys

Conclusions

- Corneal toxicity and thrombocytopenia are the main complications of therapy with AGS-16M8F and AGS-16C3F.

- Pathogenesis of both complications is still uncertain. Unlikely target-related. Our preliminary data suggest that decreased tear formation at least parallels corneal toxicity (might have a role in it?)

- Possible signs of anti- tumor effect in tumors with moderate to high expression of ENPP3.

- Resistance to anti-ENPP3 ADCs might result from growth of target-negative tumor cells.