Repair-Resistant Aristolochic Acid DNA-Adducts

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DNA Repair Interest Group
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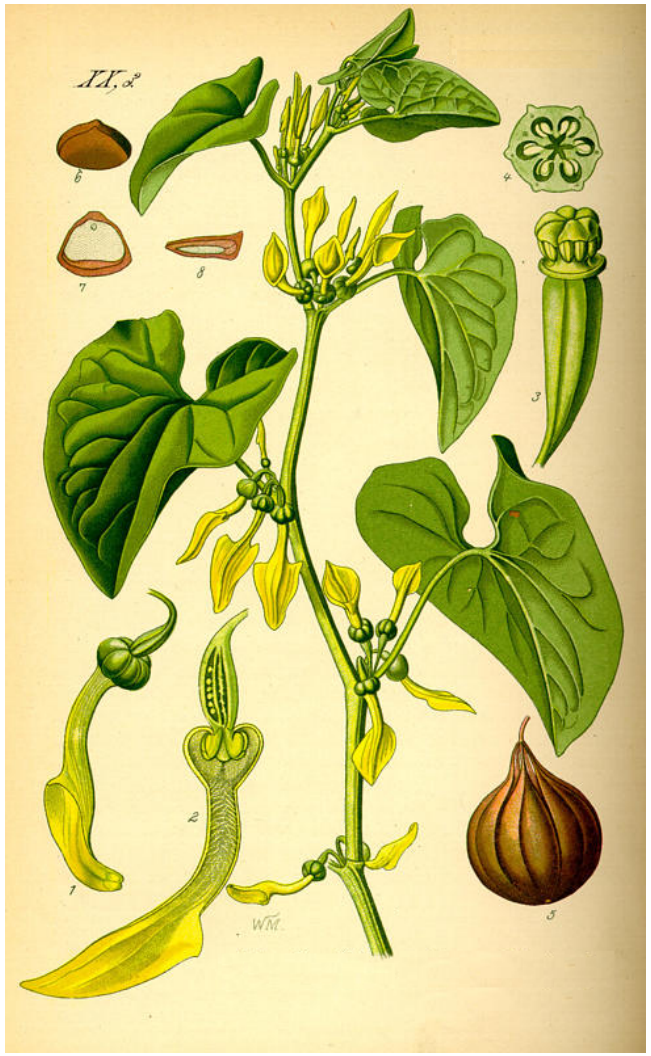
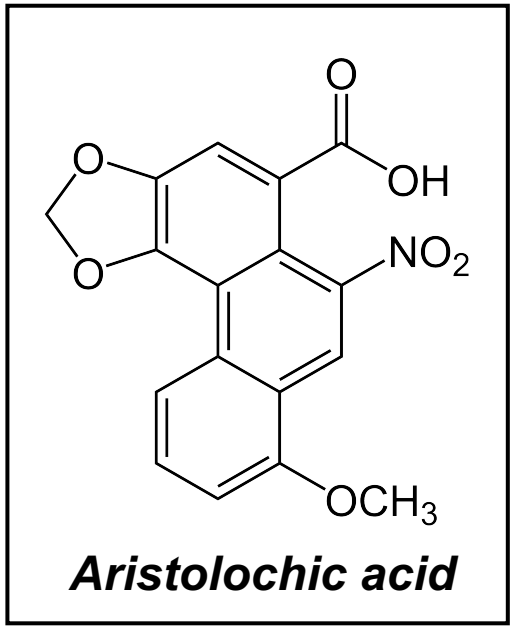
Stony Brook Medicine



Aristolochia clematitis



Carcinogen and nephrotoxin

Dioscorides, Galen, Ayuverdic medicine, printed herbals, *materia medica*, and official Pharmacopaeia promoted the use of *Aristolochia* herbs for 2500 years.

no toxicities reported

"After the incision, prepare silphium juice a drachma in weight, grate aristolochia to the amount of a deer's vertebra, and sift a half-choinix each of parched lentils and vetches…"

Hippocrates: Internal Affections (~400 BC)

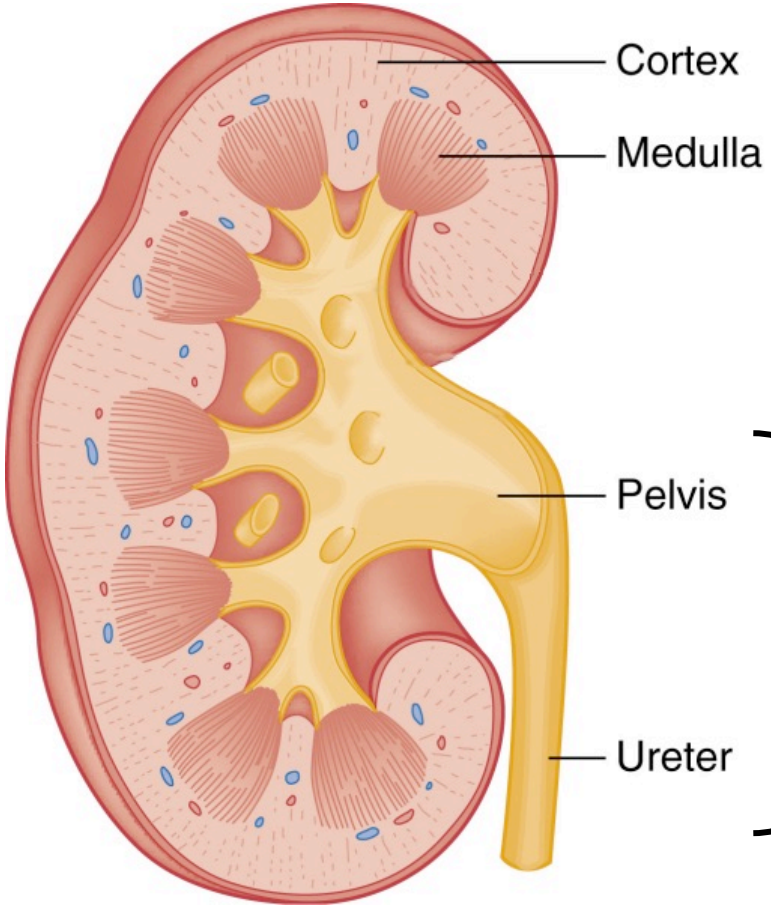
Outbreak of Renal Failure and Urothelial Cancer in Belgium

Vanherweghem *et al*., *Lancet (1993)*

- 128/1800 affected.
- >50% patients with urothelial dysplasia
- All had attended the same weight loss clinic, used a mixture of drugs and plant extracts over 18 months.
- Substitution of *Aristolochia* for *Stephania* due to translation error.

Aristolactam-DNA adducts found in renal tissue of all affected patients tested. *Schmeiser, et al (1996*)

AA exposure causes two diseases:
Renal nephropathy and/or cancer

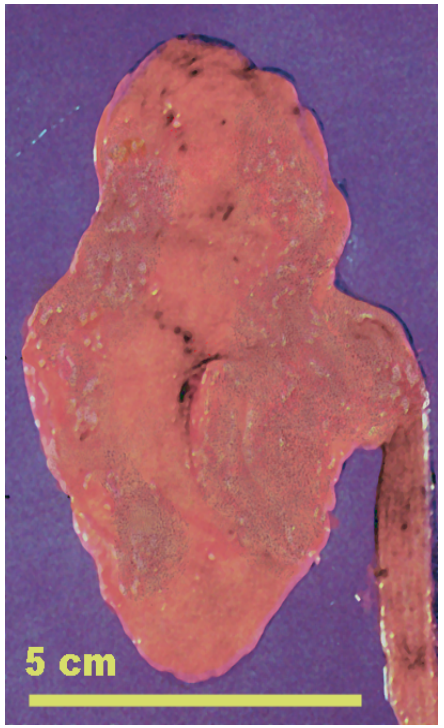


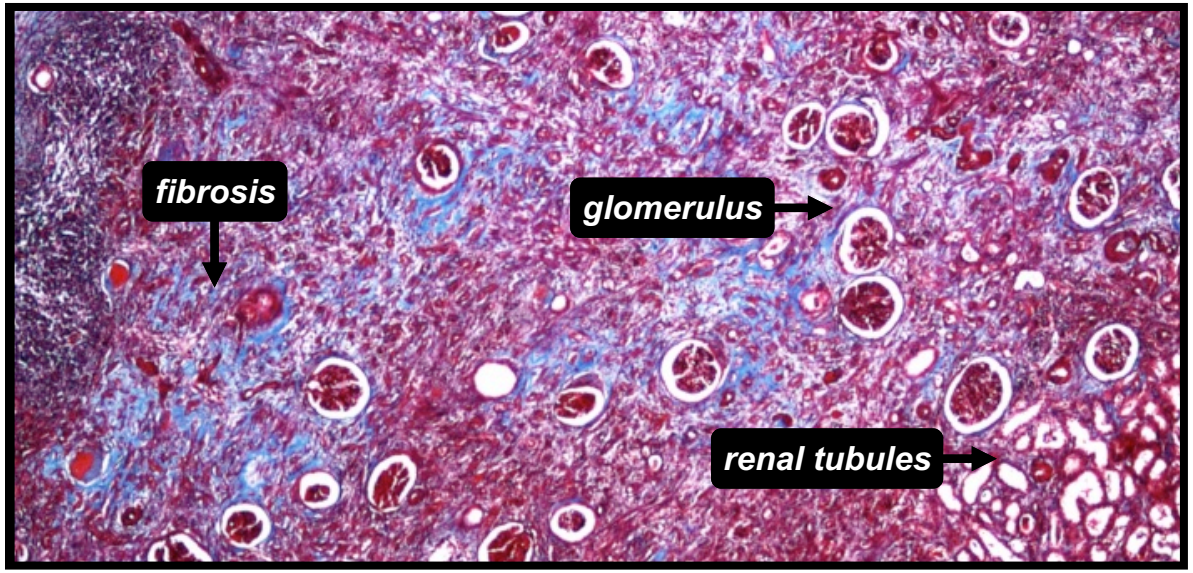
renal tubule cells → toxicity, leading to fibrosis and renal failure

upper urothelium → cancer

Pathophysiology of AAN

Chronic nephropathy with renal fibrosis, progressing invariably to end-stage renal failure.





Balkan Endemic Nephropathy (BEN)

- Occurs only in farming villages in Croatia, Bosnia, Serbia, Romania and Bulgaria.
- Geographical distribution unchanged after 50 years.
- Affects adults - often in the same household - but never children < age 18.
- ~100,000 at risk.
- Tubulointerstitial CKD (fibrosis gradient, little inflammation, sparing of glomeruli).
- Upper urinary tract cancer frequency 20X higher in endemic sites.

Dietary contamination with AA

Hranjec et al CMJ (2005)

10/24 flour samples from family farms in Croatia and Bosnia endemic areas contain *A. clematitis* DNA.

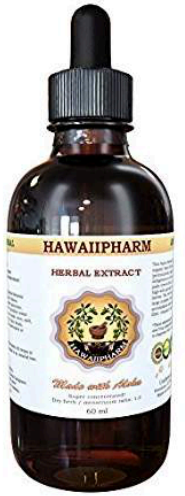
~8,000,000 at risk

Taiwan

- Highest rate of UTUC (~4 per 100K)
- Between 1997 and 2003, ***one of three*** Taiwanese were prescribed herbal remedies containing *Aristolochia* *(Wang et al, JNCI 2009)*
- *Aristolochia* still widely used in medicine and folk remedies in China, India, etc.
- 100 million at risk

Aristolochia-containing products still available on internet

ARISTOLOCHIA CLEMATITIS 30C MD
by Boiron



California Poppy (Eschscholzia Californica) and Birthwort (Aristolochia Clematitis) Liquid Extract 2 oz by HawaiiPharm
by HawaiiPharm

Awareness of AAN/UUC

100 AAN/ UUC….1800 at risk

~100,000 at risk

~8,000,000 at risk

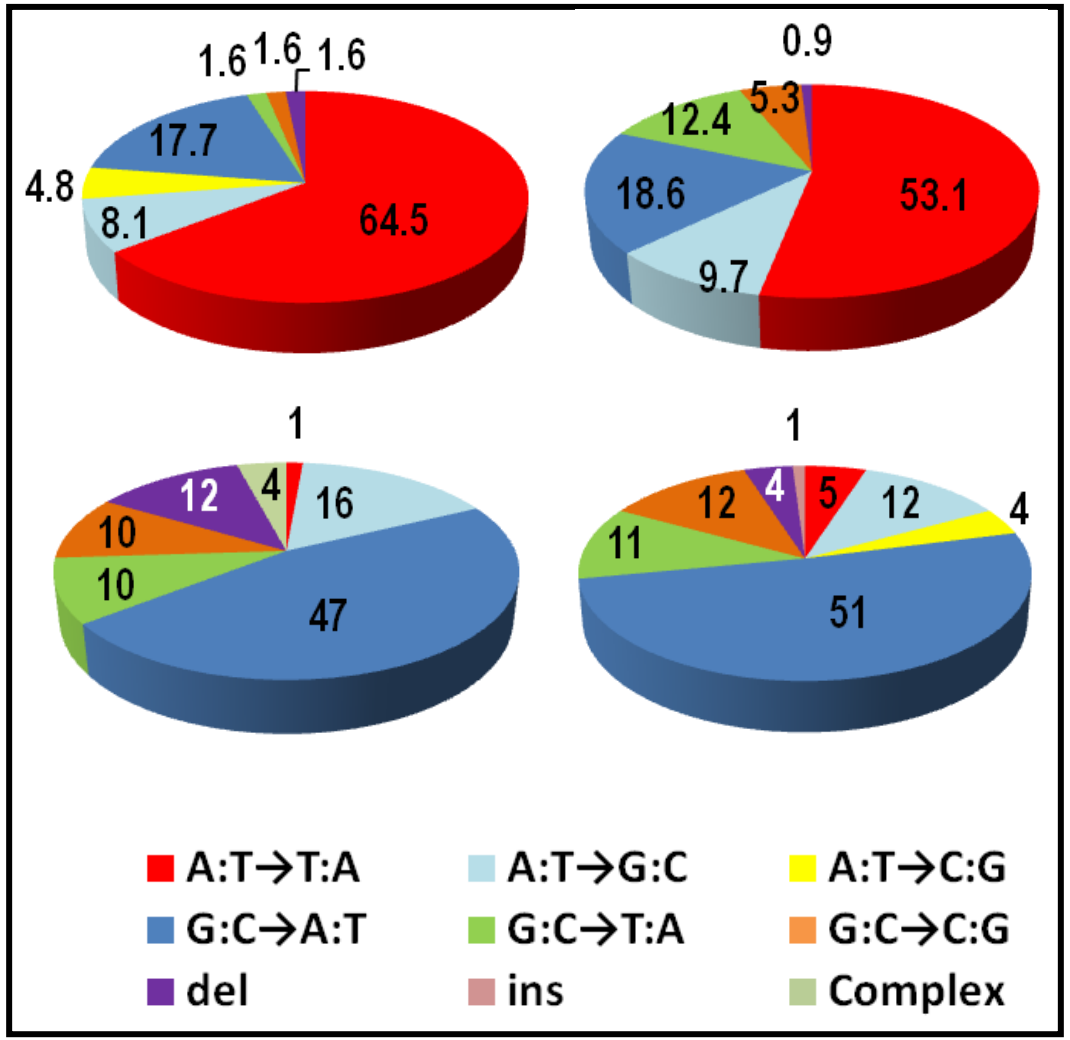
~100,000,000 at risk ?

Biomarkers of AA-exposure

- Tumor mutations
  - Tumor “driver” mutations
  - Tumor “mutational signature”
- AA-derived DNA adducts

Mutational Spectrum of the *TP53* Gene in AA-associated UTUC

Balkan UTUC 59 mutations



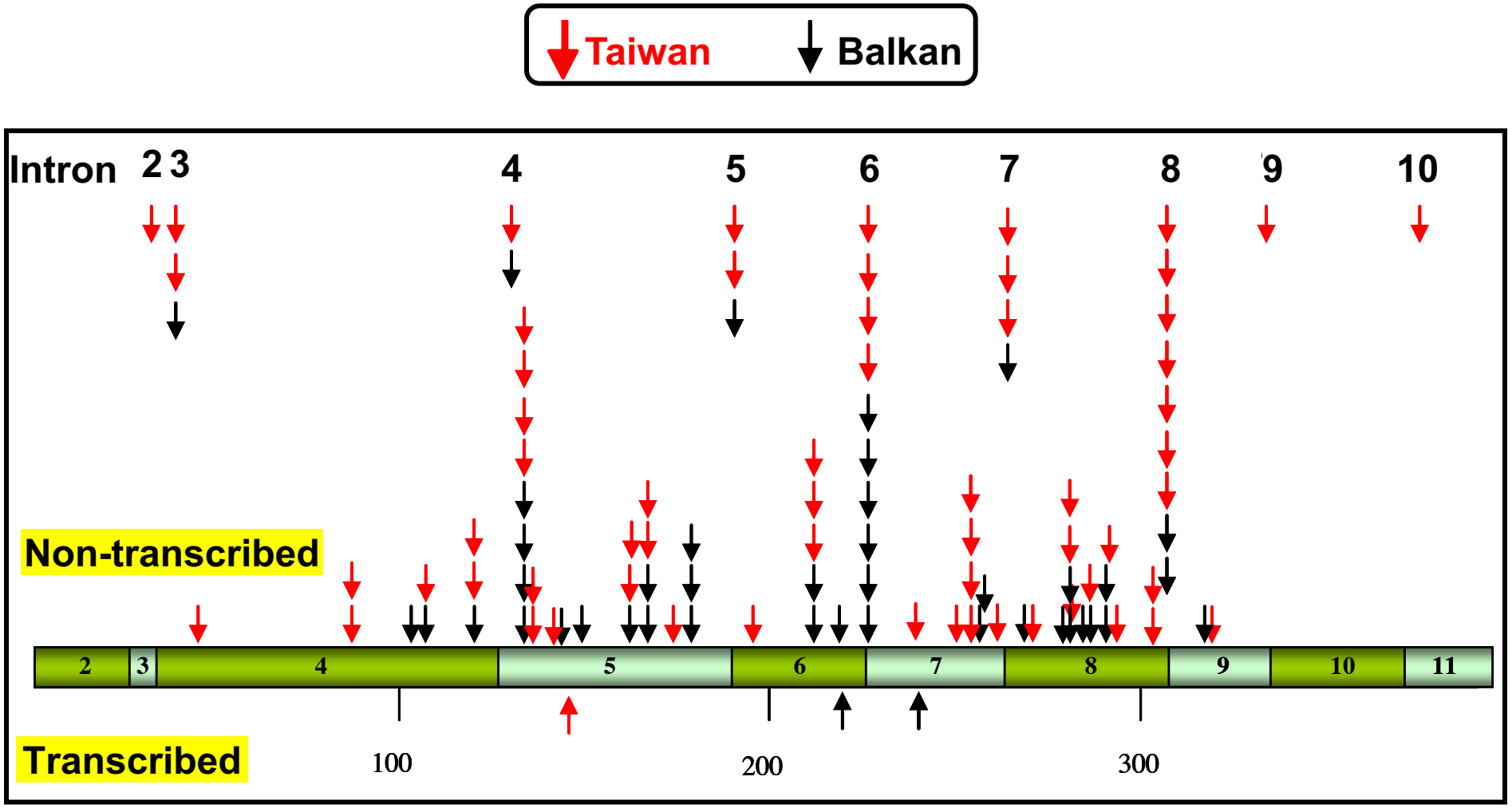
Taiwan UTUC 84 mutations

Worldwide UTUC 73 mutations

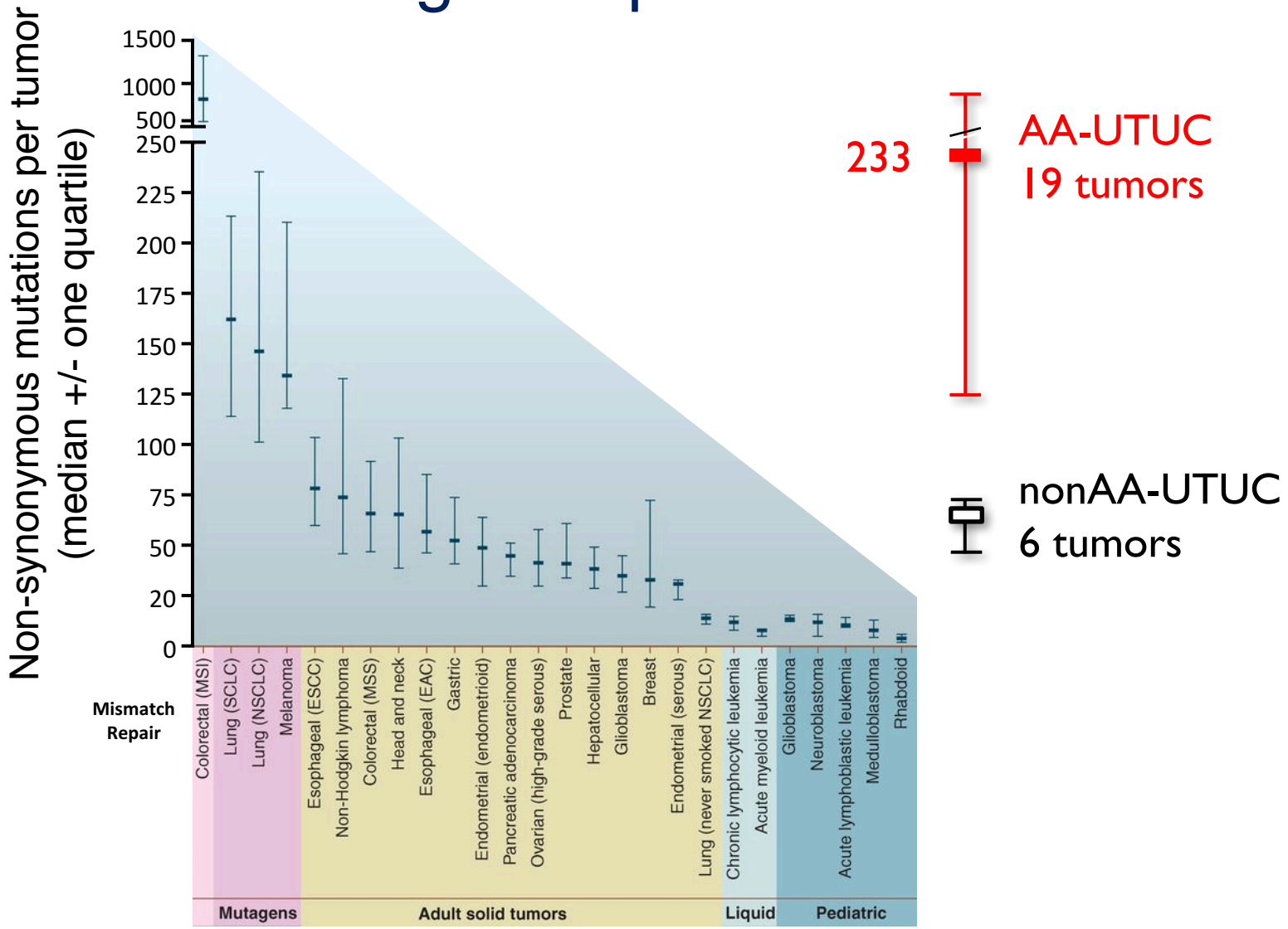
Worldwide Bladder Cancer 696 mutations

Chen *et al*., PNAS 2012

Location and Strand Specificity of A:T→T:A Transversions in the *TP53* Gene

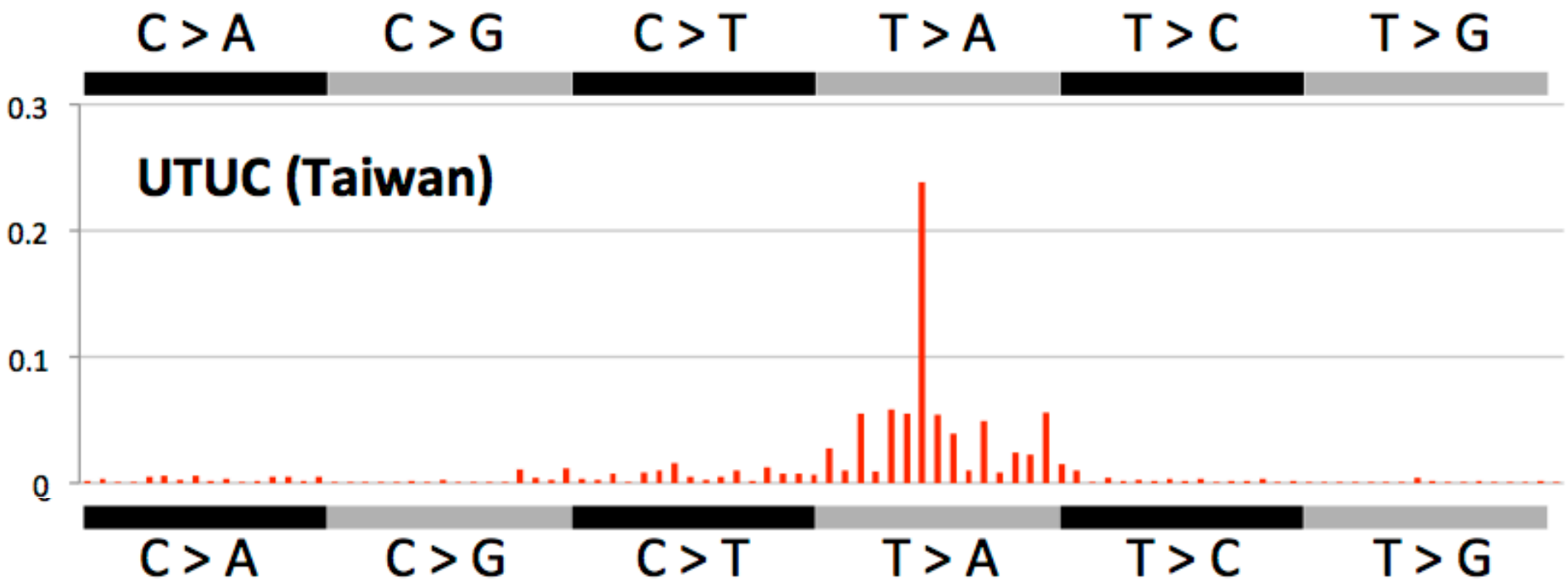


High mutational load consistent with mutagen exposure



modified from Vogelstein et al., Science 2013

UTUC mutational signature of AA

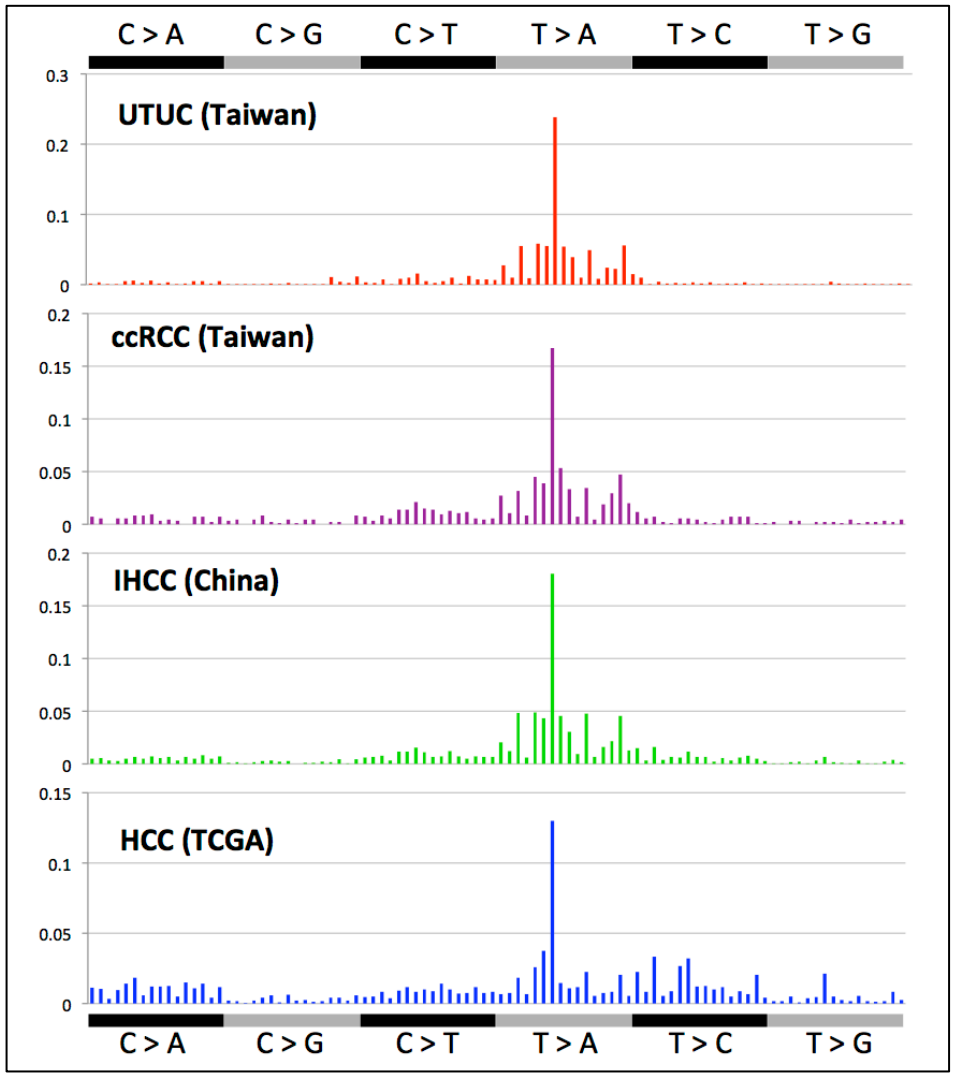


Signature #22 in COSMIC database

Genomic AA mutational signature in UTUC

- A >T transversions (>35% of all single base substitutions).
- Marked strand bias in transcribed genes with mutated adenine residues on non-transcribed strand.
- Preference for (C/T)**A**G context.
- Excess of splice acceptor site mutations.

Mutational signatures suggest AA-involvement in several cancers



	Correlation with UTUC Signature
ccRCC (Taiwan)	0.982
IHCC (China)	0.987
HCC (TCGA)	0.873

UTUC – upper tract urothelial carcinoma, BC – bladder carcinoma, RCC – renal cell carcinoma,
HCC – hepatocellular carcinoma, IHCC – intrahepatic cholangiocarcinoma

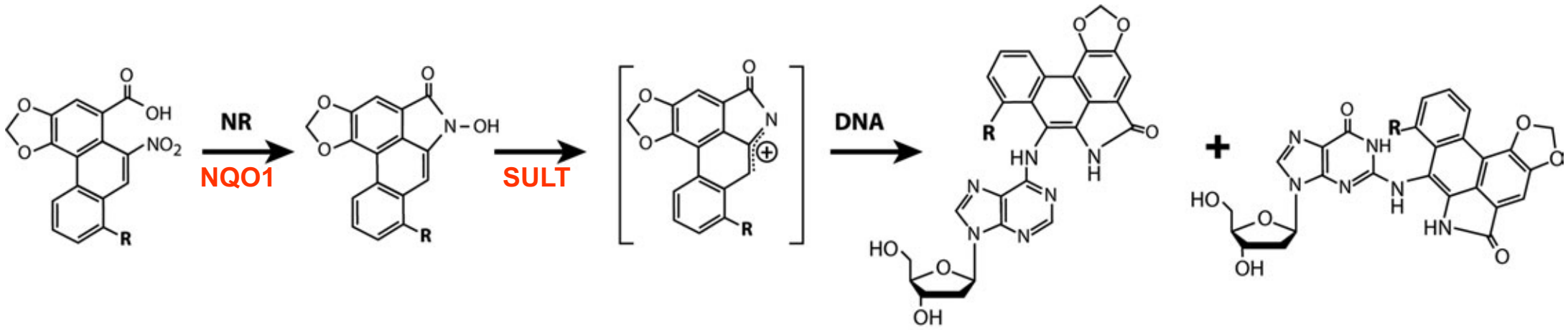
Rosenquist & Grollman, *DNA Repair*, 2016

Cancers with AA-mutational signature

Cancer	Cohorts	Mutations in Driver Genes?	References
UTUC	Taiwan Croatia	Yes	*Grollman, et al;* *Poon, et al;* *others*
BC	Taiwan Singapore China	Yes	*Poon, et al;*
RCC	Taiwan Romania Croatia	No	*Hoang, et al;* *Scelo, et al;* *Jelakovic, et al.*
HCC	China Japan Southeast Asia US	Yes	*Poon, et al;* *Totoki, et al;* *Ng, et al;* *others*
IHCC	China	Yes	*Zou, et al.*

UTUC – upper tract urothelial carcinoma, BC – bladder carcinoma, RCC – renal cell carcinoma, HCC – hepatocellular carcinoma, IHCC – intrahepatic cholangiocarcinoma

Following metabolic activation, aristolochic acid reacts with DNA (and proteins) to form covalent adducts in target tissues

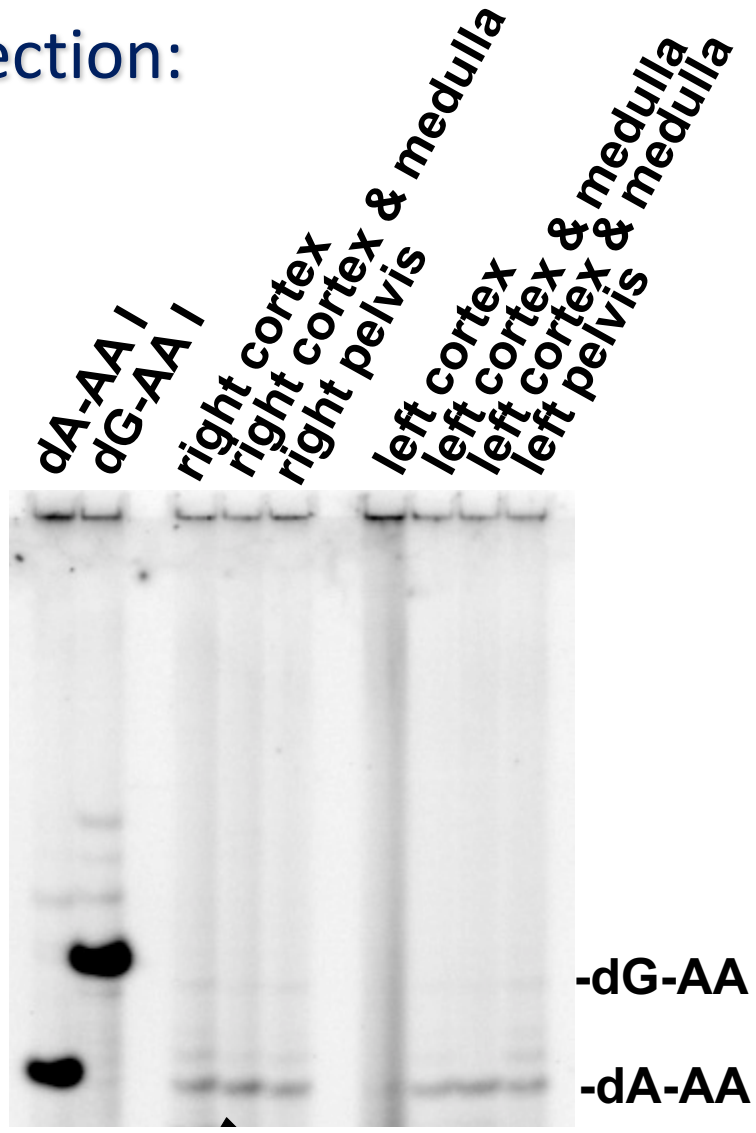


AA-I: **R** = OCH_3
AA-II: **R** = H

dA-AL - I, **R** = OCH_3
dA-AL -II, **R** = H

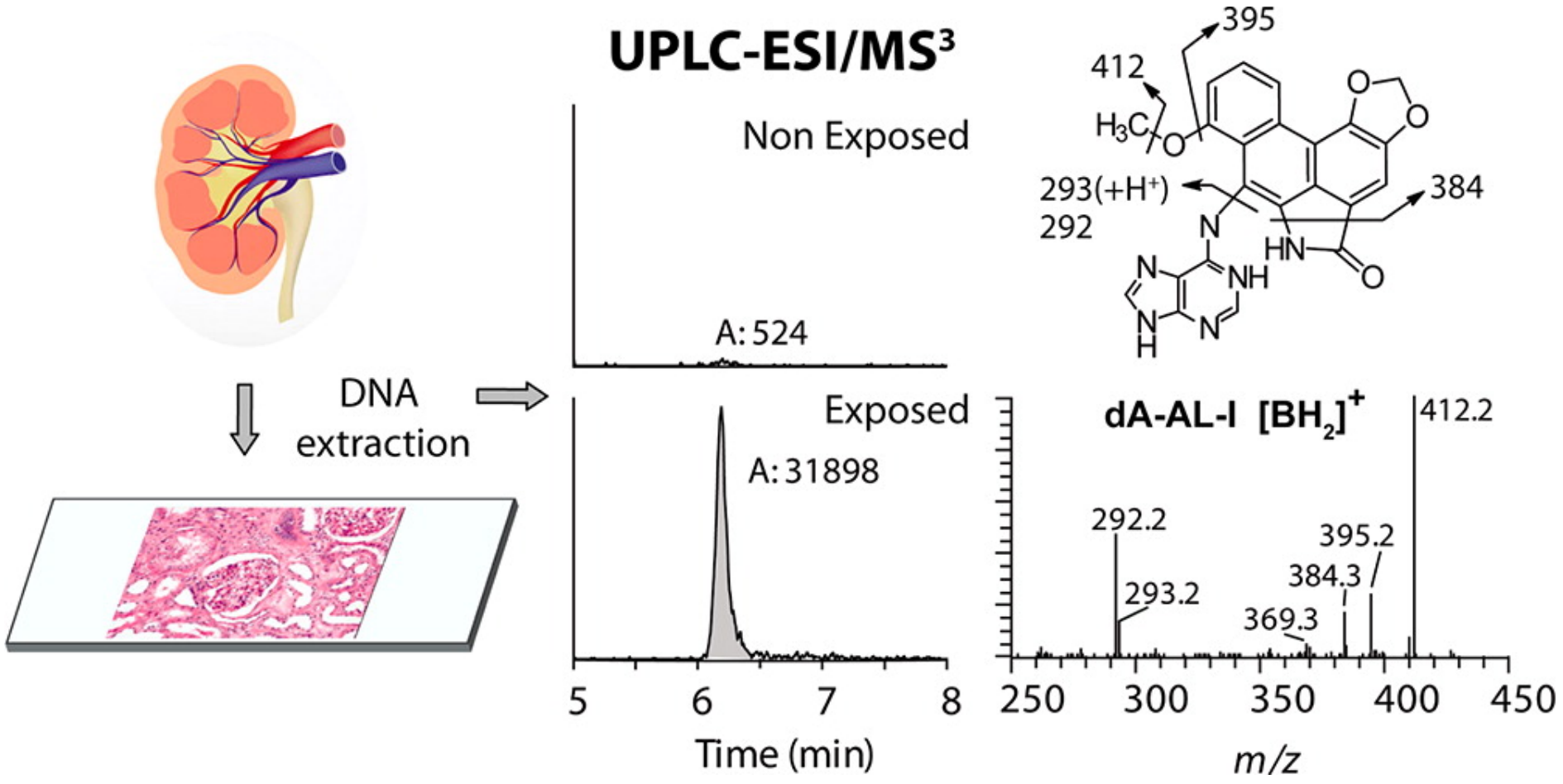
dG-AL- I, **R** = OCH_3
dG-AL-II, **R** = H

AL-DNA adduct detection:
^{32}P post-labelling



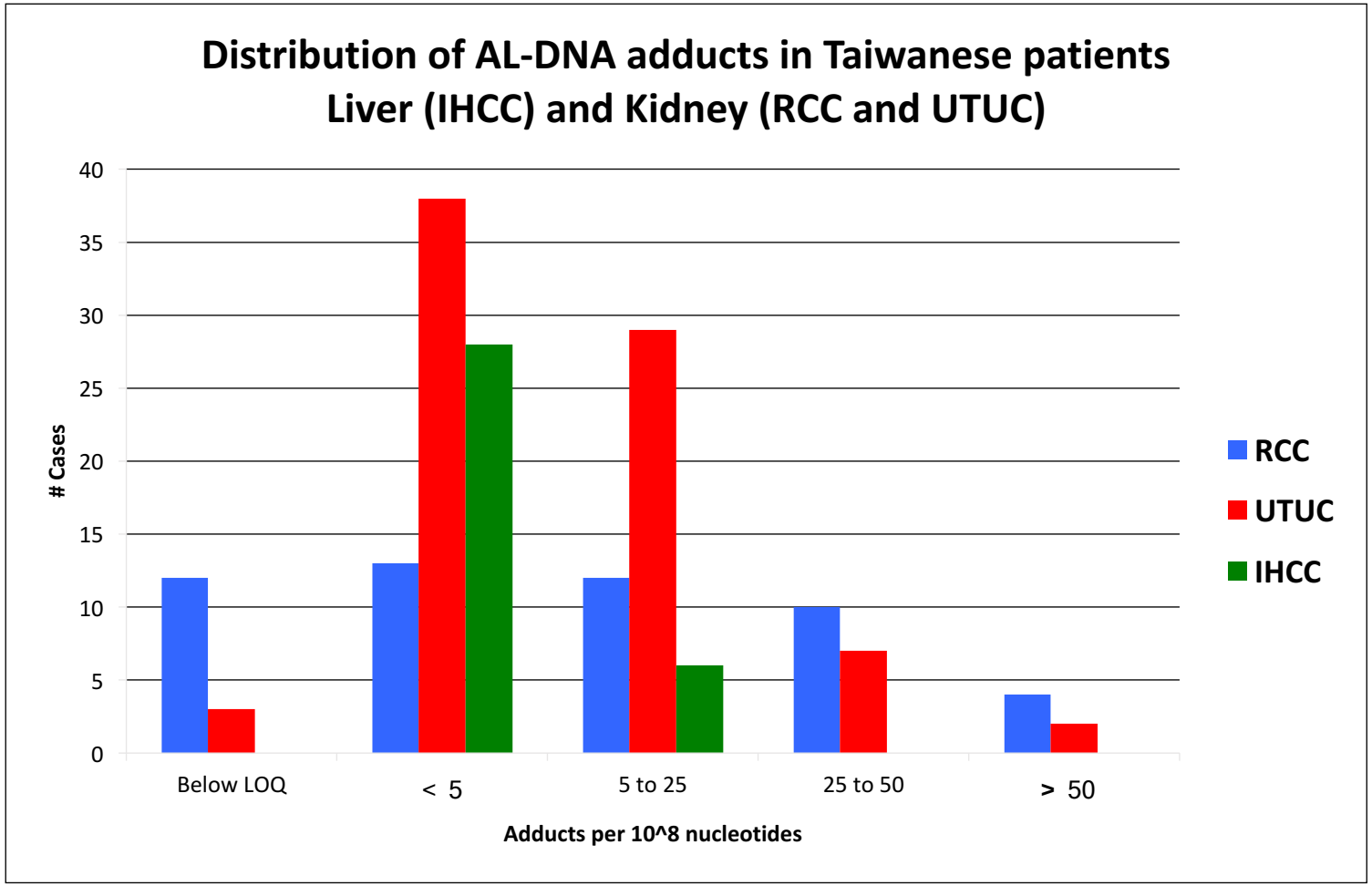
AAN patient ~3 yrs after exposure

AL-DNA adduct retrieval from formalin-fixed, paraffin-embedded human kidney

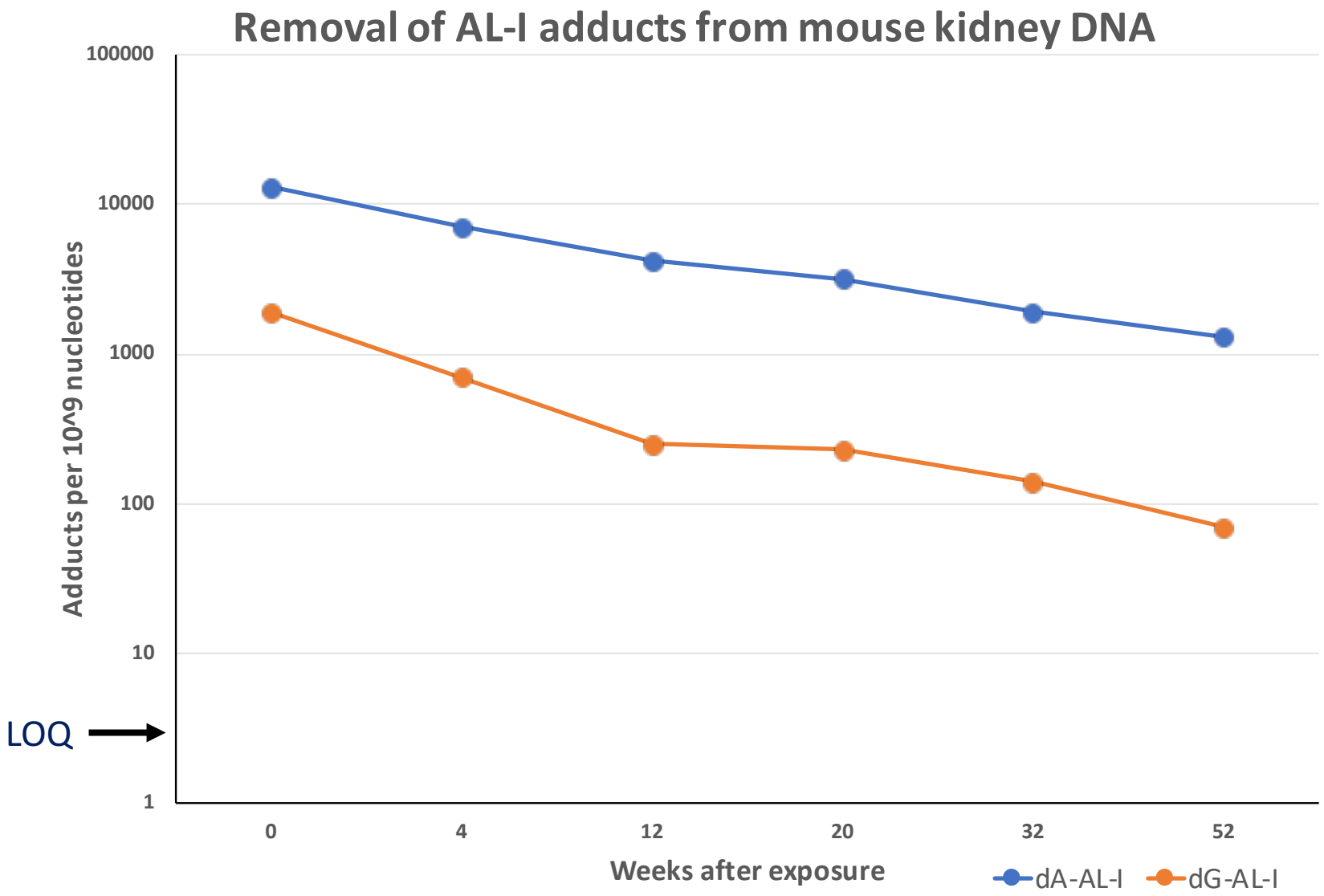


LOQ – 5 adducts per 10^9 nucleotides

AA-adducts in Taiwanese cancer patients

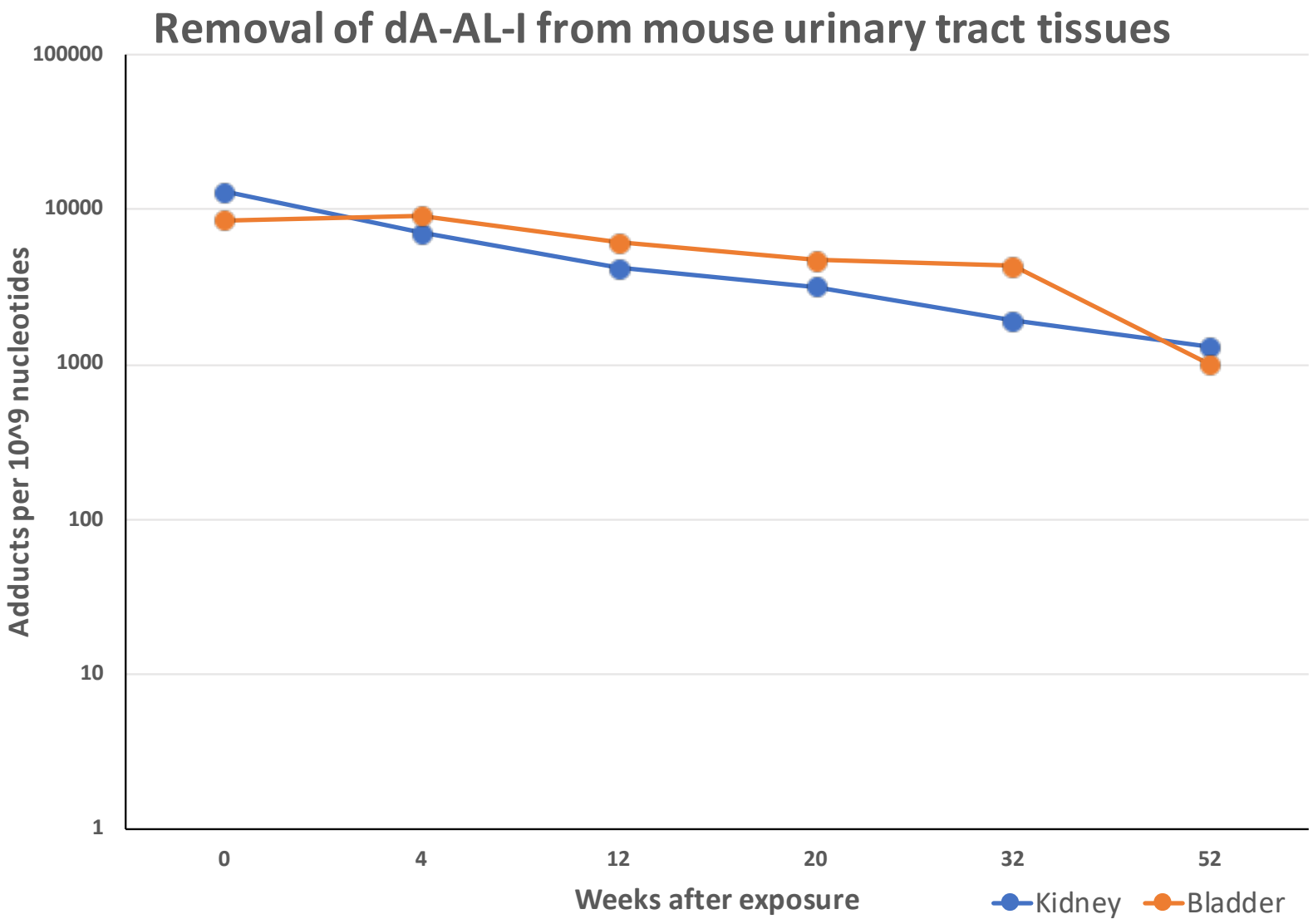


Renal cell carcinoma (RCC)
Upper tract urothelial carcinoma (UTUC)
Intrahepatic cholangiocarcinoma (IHCC)



Mice fed 6.7 ppm AAI for 1 month then switched to normal diet.

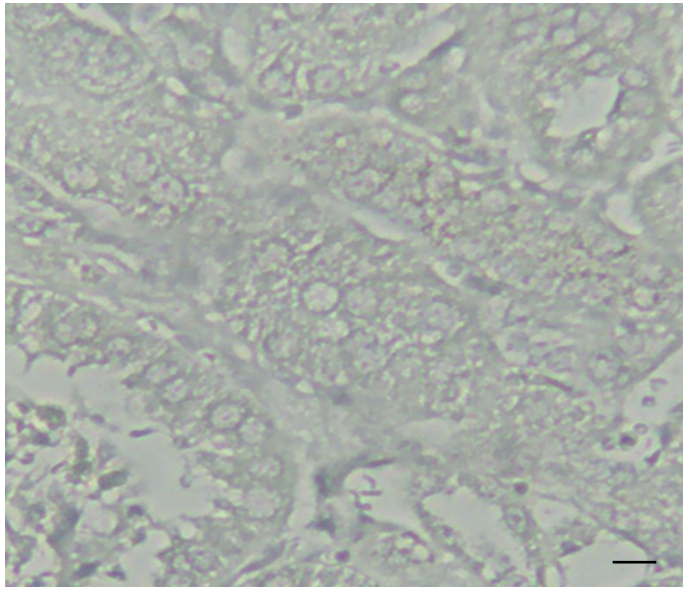
Shibutani, et al., unpublished



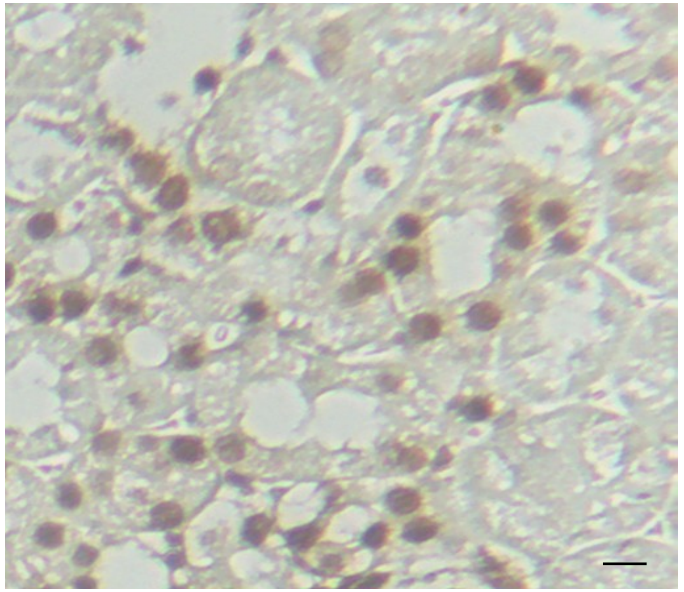
Mice fed 6.7 ppm AAI for 1 month then switched to normal diet.

Shibutani, et al., unpublished

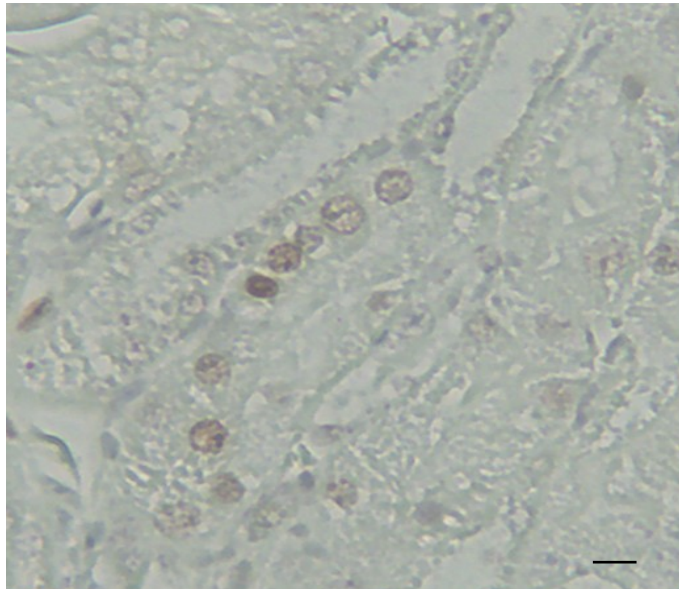
Long-term persistence of AA-adducts in mouse kidneys after AA exposure



Untreated



1 month



1 year

Immunolocalization of AA-adducts in mouse kidney.

- Rabbit monoclonal antibodies
- Immunogen was a mixture of ALI-DNA and ALI-acetylated albumin

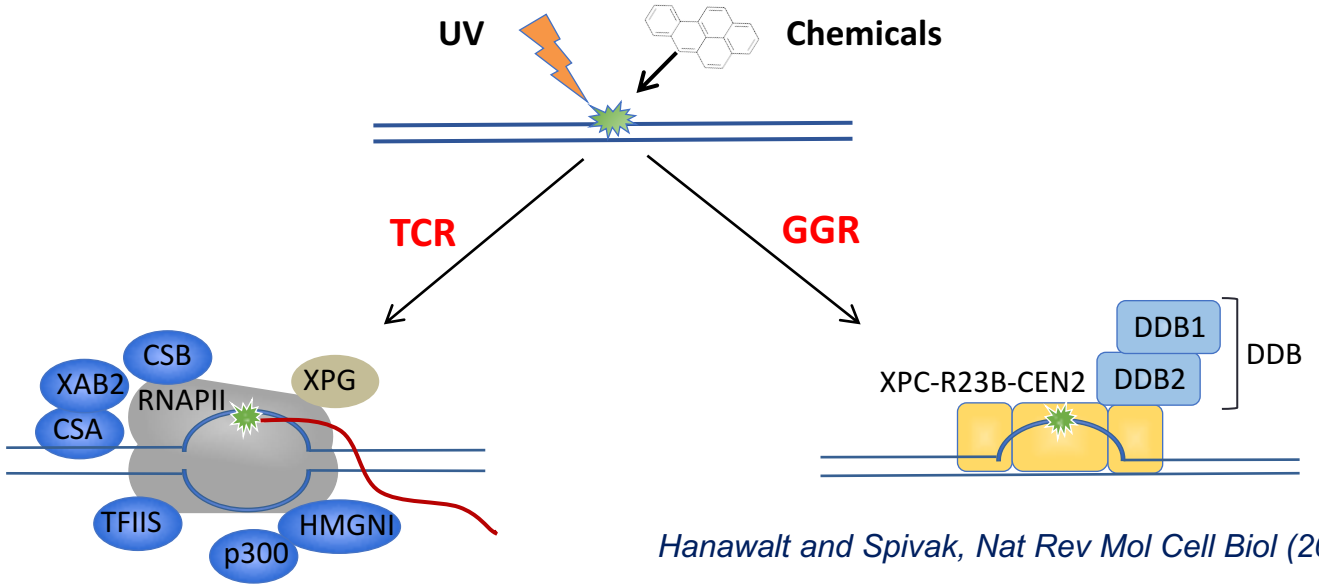
Persistence of AL-DNA adducts in human clinical samples

Schmeiser, et al., Int J Cancer (2014)

- 11 Belgian patients
- Time from known exposure to surgery 9.7 years to 20.8 years
- Renal DNA analyzed by ^{32}P post-labelling
- 11/11 patients had dA-AL-I adducts

Aristolactam-DNA adducts are resistant to global genomic repair

Sidorenko, et al. NAR, 2012

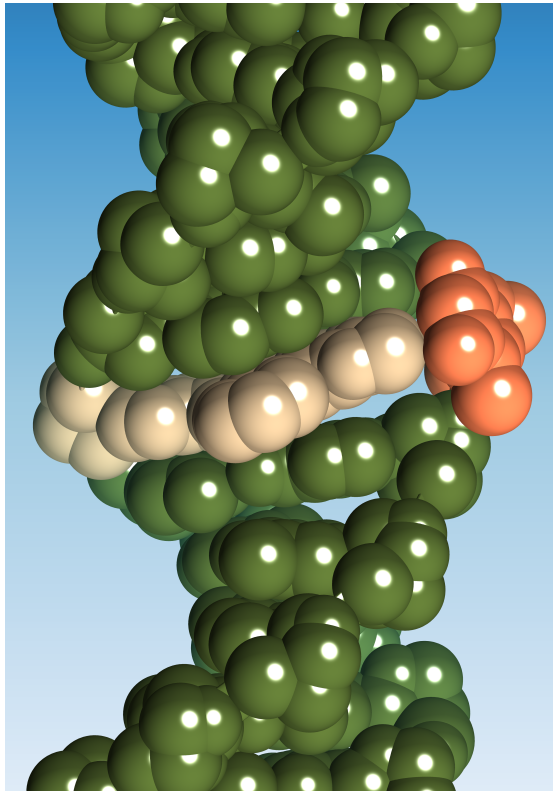


Transcription arrest, factor recruitment

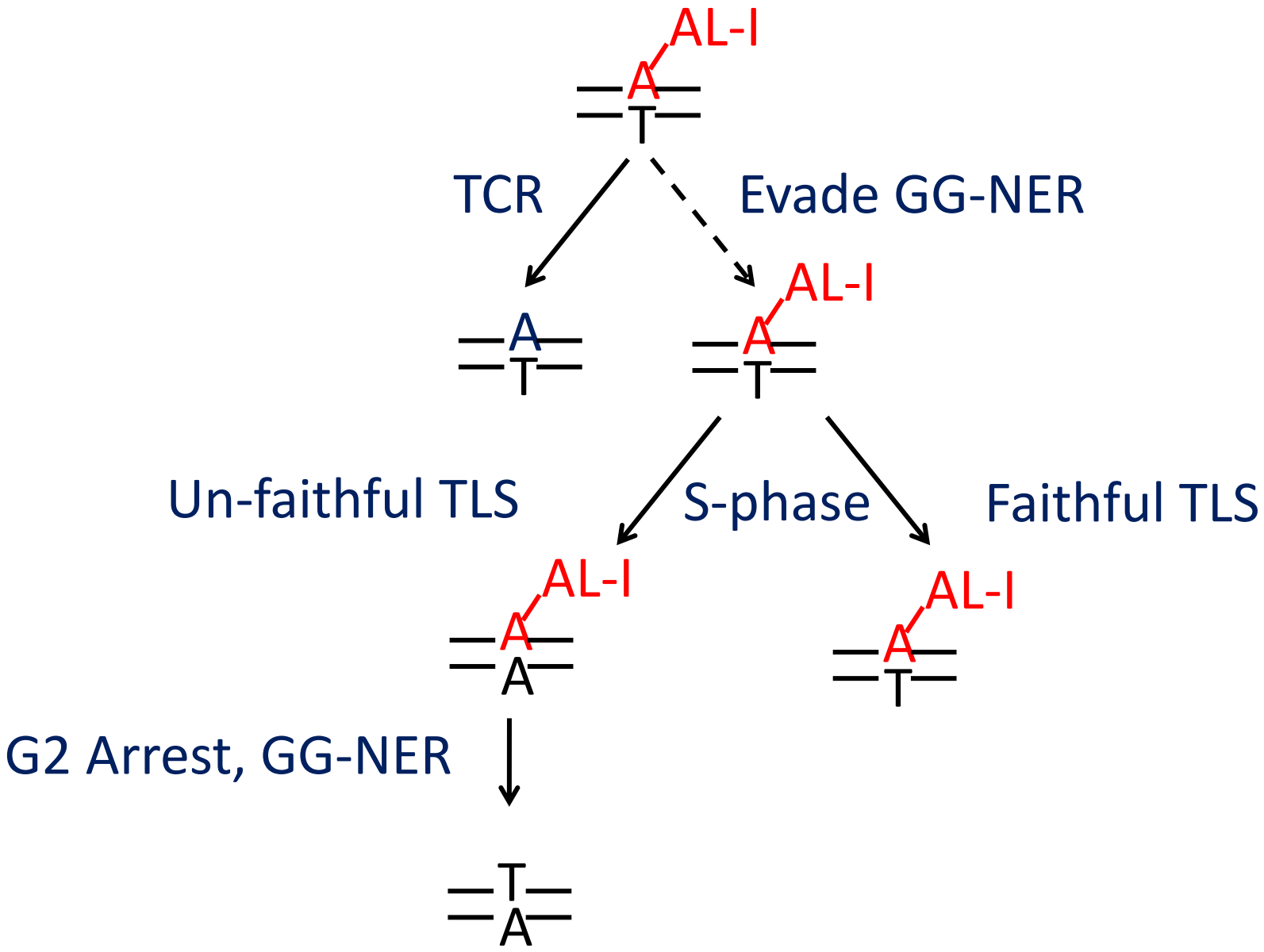
Lesion recognition

Hanawalt and Spivak, Nat Rev Mol Cell Biol (2008)

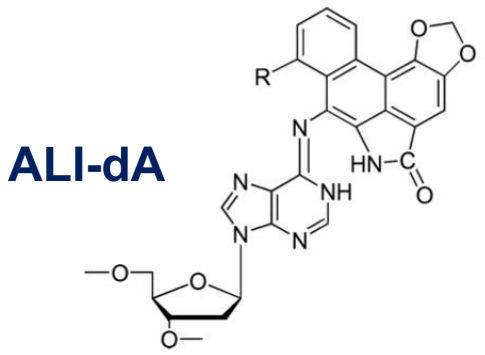
AL-DNA adducts do not significantly perturb DNA structure



Lukin, et al., NAR, 2012



Adapted from Rosenquist & Grollman, *2017*



Aristolactam-DNA Adducts

- ALI-dA is pro-mutagenic – leads to A→T transversion mutations.
- Removed by transcription-coupled repair, ***resistant*** to global genome DNA repair.
- Can be detected in genomic DNA decades after exposure.
- Sensitive methods for detection (**excellent biomarker**)
  - ^{32}P-postlabeling, ~2 adduct per 10^8 nucleotides
  - UPLC-MS^3, ~ 3 adducts per 10^9 nucleotides

What are the roles of aristolochic acid in carcinogenesis?

Cancer	Evidence of Exposure	Mutations in Driver Genes?	
UTUC, BC	Epidemiology Adducts Mutational Signature	Yes	*Grollman, et al;* *Poon, et al;* *others*
RCC	Epidemiology Adducts Mutational Signature	No	*Hoang, et al;* *Scelo, et al;* *Jelakovic, et al.*
HCC	Adducts Mutational Signature	Yes	*Poon, et al;* *Totoki, et al;* *Ng, et al;* *others*
IHCC	Adducts Mutational Signature (China-yes, Taiwan ?)	Yes	*Zou, et al.*

Summary

- Aristolochic acid is implicated in the etiology of urothelial, renal, liver, and biliary tumors.
- AA-associated tumors marked by high mutation rate and unique-mutational signature.
- AL-DNA adducts evade global genome repair.

Current questions

- Are persistent AL-DNA adducts an ongoing source of mutations?
- Where in the genome are persistent DNA-adducts located?

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