For Science Issue 6
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Mode of Action for Inorganic Arsenic Carcinogenesis

- Key Events
  - Ingestion of significant amounts of arsenic
  - Generation of trivalent forms ($InAs^{III}$, MMA^{III}, DMA^{III})
  - Concentration of reactive trivalent arsenicals in target tissue
  - Reaction with critical cellular thiols (glutathione, proteins)
  - Cytotoxicity and cell death
  - Regenerative proliferation
  - Tumors

Comparison of Cytotoxicity of Trivalent Arsenicals for Various Cell Types

Cells	LC_{50} Concentration (μM) iAs^{III}	MMA^{III}	DMA^{III}
HBEC (human bronchial)	5.8	1.0	1.4
HEK001 (human keratinocytes)	19.0	1.6	1.7
1T1 (human urothelial)	16.7	2.1	1.7
MYP3 (rat urothelial)	2.9	1.0	1.9

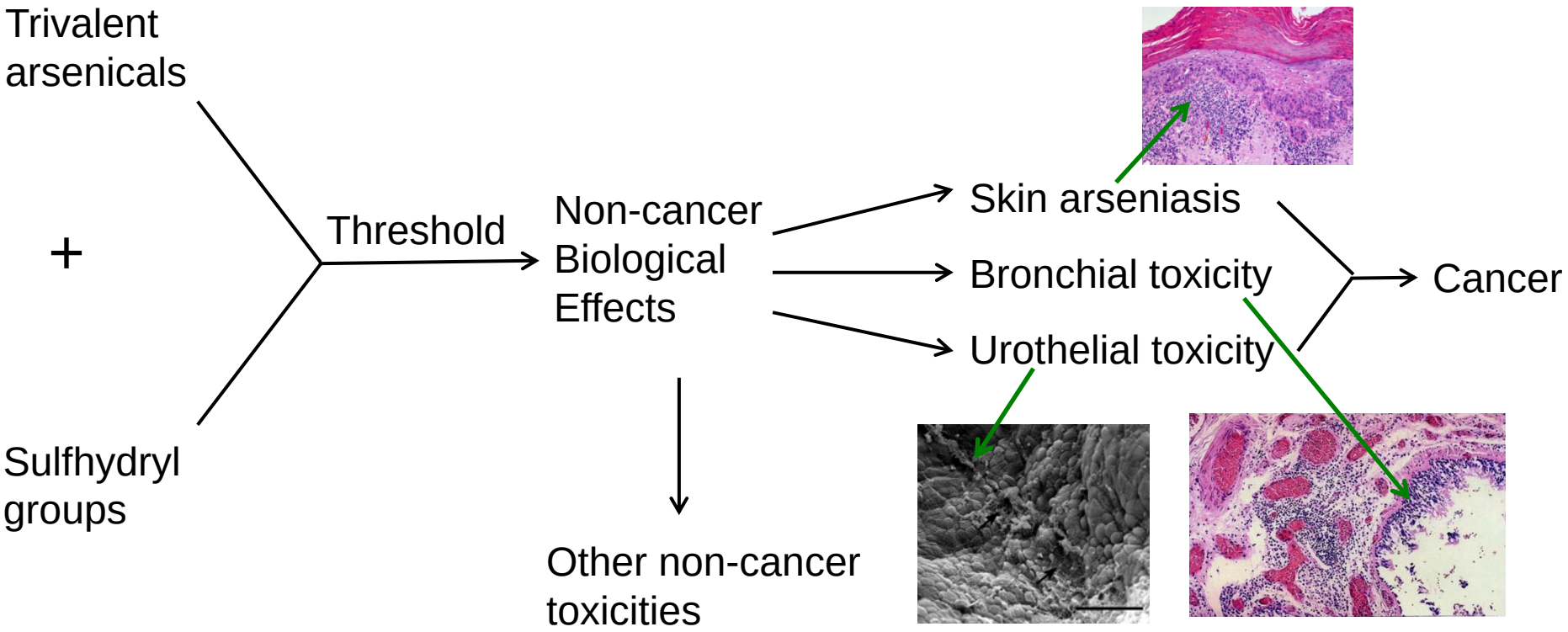
Cytotoxicity of $DMMTA^{V}$

- LC_{50} for rat MYP3 urothelial cells 1.3µM
- LC_{50} for human 1T1 urothelial cells 1.4µM

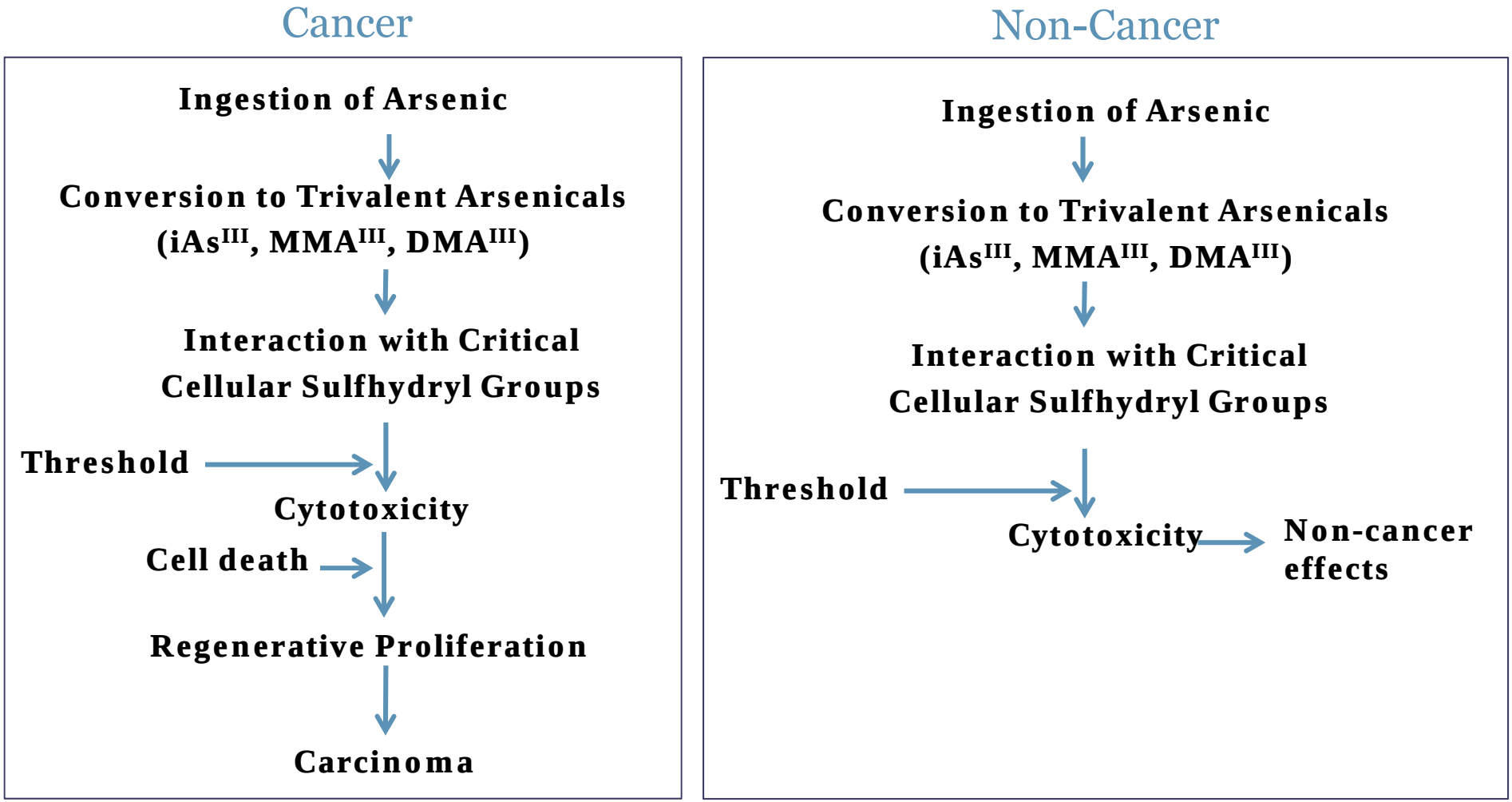
- $DMMTA^{V}$, in contrast to DMA^{V}, rapidly enters cells
- $DMMTA^{V}$ rapidly converts to DMA^{III} once inside cell

- Critical cytotoxic agent is DMA^{III}

Implications for Risk Assessment



Mode of Action for Inorganic Arsenic: Working Hypothesis



Research Plan

4) Human *Drinking Water Concentration*

Pharmacokinetic variability
(Drinking water to urine ratio *in vivo)*

Weighted average BMDL
for total arsenic in urine

Equivalent urine concentration
for mixtures

In vitro BMDLs for
trivalent/pentavalent mixtures

3) Human *in vitro*

Uroepithelial cells

2) Mouse *in vivo*

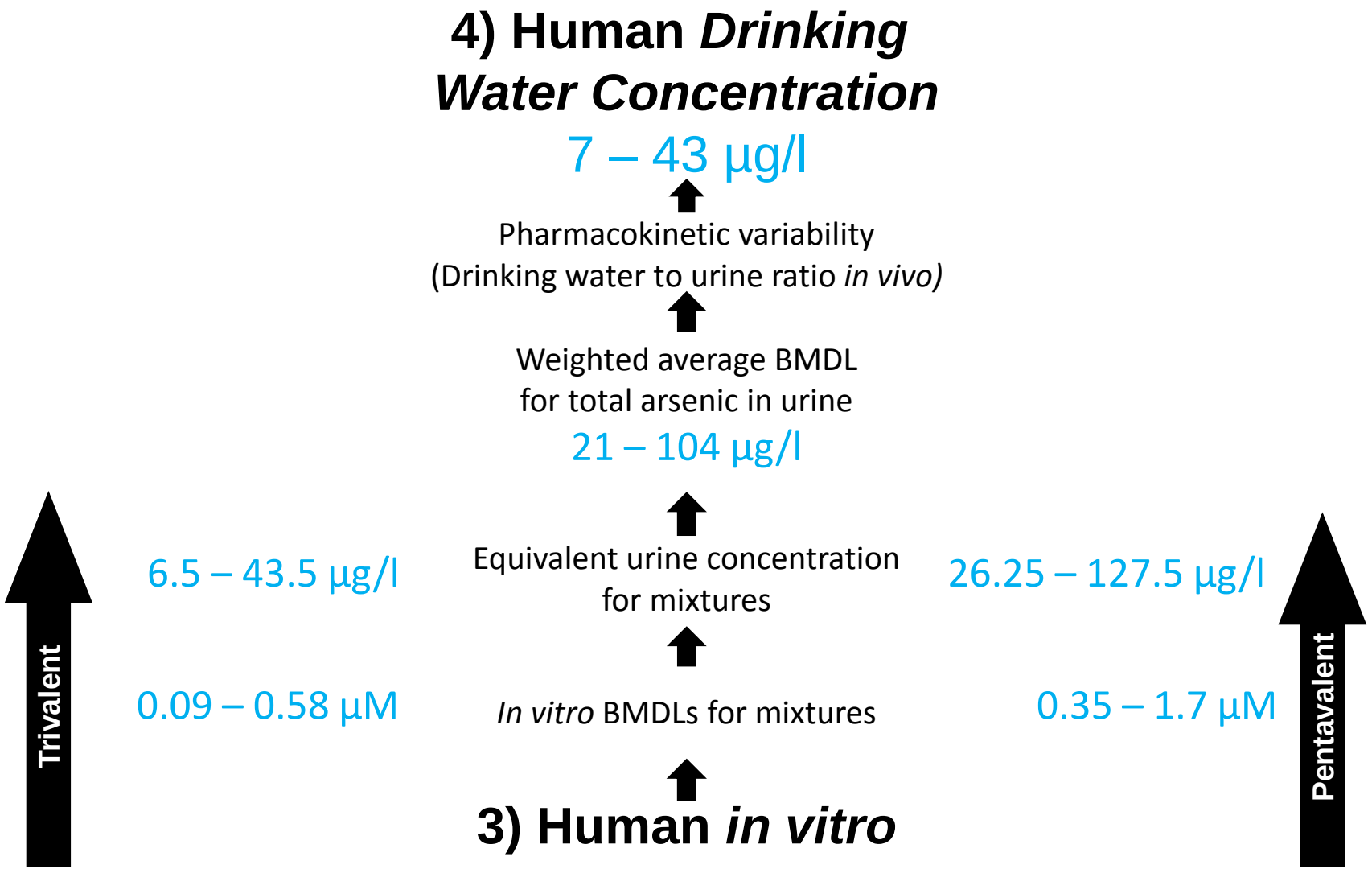
Bladder genomics

Validate ability of *in vitro* studies to predict *in vivo* response

***1) in vitro* data**

Literature

Definition of a Point of Departure



Pathway categories for As mixture for different cell types	Number of Possible Total Pathways in Categories	Number of Pathways in Categories for Lung	Median BMDs Lung	Number of Pathway in Categories for Uroepithelial	Median BMDs Uroepithelial
	Possible	Week 1	Week 1	Week 1	Week 1
Apoptosis and survival	35	35	3.20 – 4.91	35	3.47 – 5.33
- p53 dependent apoptosis	1	1	3.88	1	4.23
- caspase cascade	1	1	3.97	1	4.29
Cell adhesion	18	18	2.97 – 4.35	18	3.91 – 4.71
Cell cycle	17	17	2.75 – 4.54	17	2.61 – 5.25
- regulation of G1/S transition	3	3	3.52 – 3.99	3	3.58 – 4.43
Chemotaxis	7	7	3.38 – 4.23	7	3.91 – 4.91
Cytoskeleton remodeling	19	19	2.47 – 4.75	18	3.58 – 4.73
Development	122	121	1.01 - 4.95	121	2.71 – 5.75
- Tgfb induction of EMT	4	4	3.03 – 4.26	4	3.33 – 4.36
- activation of Erk	6	6	3.86 – 4.41	6	4.17 – 4.36
- Egfr signaling	3	3	3.49 – 4.66	3	3.75 – 3.99
- Vegf signaling	3	3	3.13 – 4.76	3	4.07 – 4.37
- Wnt signaling	3	3	3.53 – 4.55	3	3.36 – 4.13
- Esr1	3	3	3.85 – 4.14	3	3.75 – 4.15
- Notch signaling	1	1	4.02	1	4.09
- Igf1r signaling	1	1	4.49	1	4.15
DNA damage response	11	11	2.21 – 4.65	11	3.69 – 5.18
G-protein signaling	28	27	2.15 – 4.98	28	1.55 – 4.77
Immune response (Inflammatory)	97	97	1.95 – 5.09	97	3.30 – 5.39
Oxidative stress	2	2	2.24 – 4.34	2	3.99 – 4.26
Signal transduction	11	11	3.52 – 4.66	11	3.50 – 4.57

Genomic Analysis of iAs and As_2O_3 on Treated Lung Cells

	iAs			**As_2O_3**		
Dose (µM)	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
0.06	0	1	0	1	0	0
0.18	0	0	0	5	0	0
0.60	10	20	0	80	18	2
1.80	163	258	32	628	73	171
6.00	2001	2439	2535	3267	2309	2456

One-way Anova analysis results

- ± 1.5 fold-change filter
- 0.05 p-value filter