# *Could bladder cells shed into urine be used to indicate risk of developing cancer in remote organs using Next Generation Sequencing? A focused, mixed methods review.*

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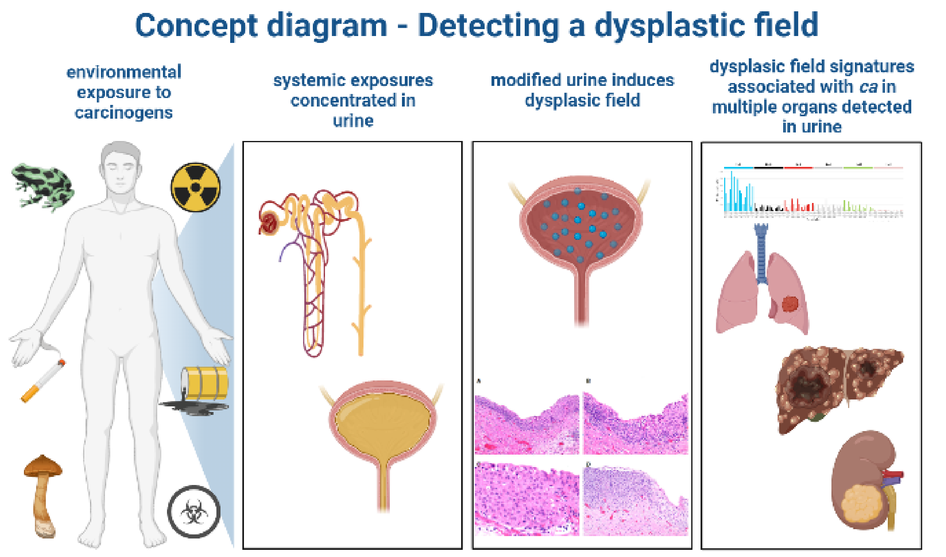
## Background:

- In 1953 Slaughter et al. hypothesized the '*field effect'* – that cancer arose from an area of 'altered' tissue.[1]
- In 1999 Kopelovich described the '*extended field effect'* – that detectable changes in a surrogate organ might indicate exposure in another organ known to be affected by the same exposure/pathway/mechanism.[2]
- Environmental exposure is reflected in the genome/dysplastic tissue.[3]
- Dysplastic tissues are already monitored in detection strategies as part of screening for cancer (e.g. Barrett's oesophagus).
- Dysplasia in the bladder can be a precursor to cancer and might potentially be detectable by testing urine. Urine offers a noninvasive way to monitor the accumulation of mutagens in the bladder epithelium.
- Epigenetic '*field cancerization*' has been successfully detected by biopsy.[4]
- Mutational signatures would be one way of measuring *exposure* if they could be derived from urine.




**Hypothesis :** ***Detectable mutational signatures from voided urine might indicate systemic exposure and inform cancer risk both in the bladder and remote organs.***

## Methods:

- Part A: In July 2022, a literature review was carried out to answer the question, ***'what studies have successfully sequenced DNA from voided urine and what were the genomic outputs?'***
- Part B: An investigation of the Signal dataset to identify mutational signatures that are common to tumors of the bladder and other organs to answer the question: ***'what are the candidate signatures and organs as surrogate/proxy targets?***

## Results part A: Literature review

- The studies listed below have successfully sequenced bladder cells from urine with deep or ultra-deep sequencing from panels of targeted genes.
- To date, no studies have sought to derive mutational signatures from bladder cells.
- The quality and quantity of DNA in voided urine is sufficient for the Next Generation Sequencing of voided bladder cells.

| | Bladder cancer | Neoplasia | Recurrence | Non muscle invasive | Muscle invasive | Healthy controls | Methylation | TERT region | Other |
|---|---|---|---|---|---|---|---|---|---|
| Kinde 2013 | ✓ | ✓ | ✓ | ✓ | | | | ✓ | |
| Ward 2016 | ✓ | | ✓ | | | ✓ | | ✓ | ✓ |
| Togneri 2016 | ✓ | | | | | | | | ✓ |
| Patel 2017 | ✓ | | ✓ | | ✓ | | | | ✓ |
| Feber 2017 | ✓ | | | ✓ | ✓ | ✓ | ✓ | | |
| Heijden 2018 | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| Ward 2019 | ✓ | | | ✓ | ✓ | | | ✓ | ✓ |
| Ward 2022 | ✓ | | ✓ | ✓ | ✓ | | | ✓ | ✓ |

## Results part B: Signature analysis

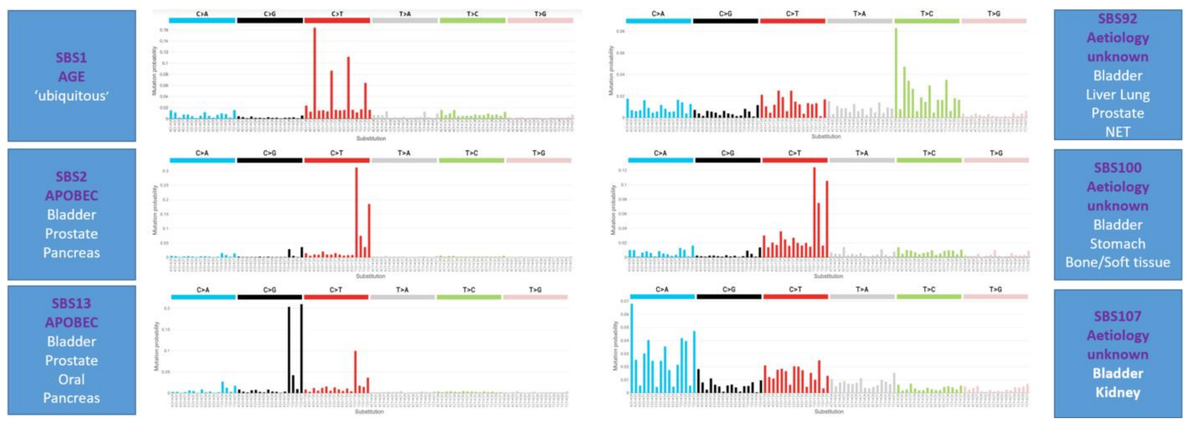
- From the Signal[5] dataset 17 signatures are identified that are shared between tumors of the bladder and other organs including two in the APOBEC pathway and two with mismatch repair (MMR) involvement.

| | Endogenous | Exogenous | Unkown |
|---|---|---|---|
| Signatures | SBS 1 age | SBS 4 smoking | SBS 17 |
| | SBS 2 APOBEC | SBS 31 platinum | SBS 23 |
| | SBS 6 MMR | SBS 94 smoking? | SBS 33 |
| | SBS 13 APOBEC | SBS 107 smoking? | SBS 57* |
| | SBS 20 MMR/POLD | | SBS 92 |
| | | | SBS 100 |
| | | | SBS 105 |
| | | | SBS 109 |

- The below heat map depicts the percentage of tumors for each organ type that contain a specific signature. The data is extracted from the Signal dataset.[5]

| | SBS 1 | SBS 17 | SBS 13 | SBS 2 | SBS31 | SBS57 | SBS 4 | SBS 6 | SBS33 | SBS92 | SBS100 | SBS23 | SBS94 | SBS20 | SBS105 | SBS107 | SBS109 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bladder | 75 | 13 | 94 | 91 | 1 | 2 | 1 | 1 | 1 | 87 | 57 | 2 | 1 | 0.29 | 0.29 | 62 | 2 |
| breast | 85 | 3 | 37 | 46 | 0.12 | 1 | 0.04 | 0.08 | 0.039 | | | 0.04 | 0.04 | | 0.04 | | |
| colorectal | 95 | 39 | 0.04 | 16 | | | 0.04 | 2 | 0.04 | | | | 0.38 | | | | |
| kidney | 77 | 0.22 | 41 | 0.04 | 0.37 | 1 | | 0.15 | | | | | | | | 92 | |
| lung | 65 | 10 | 37 | 41 | 1 | | 82 | | 0.3 | 84 | | | | | | | |
| NET | 76 | 24 | 21 | 21 | | 1 | 45 | | | 57 | 1 | | | | | | |
| myeloid | 71 | 34 | 8 | 8 | 1 | 1 | | | | | 26 | | | | | | |
| oral/oropharyngeal | 90 | 9 | 66 | 43 | 1 | | 1 | | 2 | | | | | | | | |
| ovary | 72 | 4 | 30 | 1 | 1 | 2 | | 1 | | | | | | | | | |
| prostate | 90 | 11 | 72 | 72 | | 1 | | | 0.32 | 72 | | | | | | | |
| bone and soft tissue | 78 | 11 | 38 | | 1 | 2 | | | | | 59 | | | | | | |
| pancreas | 88 | 20 | 51 | 51 | | 2 | | 1 | | | | | | | | | |
| skin | 29 | 60 | 12 | 0.39 | | | | | | | | 0.39 | | | | | |
| uterus | 85 | 13 | 16 | 42 | 0.28 | | | | | | | | | 1 | | | |
| CNS | 80 | 0.23 | 1 | 1 | | | 1 | | | | | | | | | | |
| biliary | 77 | 46 | 50 | 38 | | | | | | | | | | | | | |
| stomach | 69 | 73 | 27 | | | | | | | | 63 | | | | | | |
| liver | | | | | | | 86 | | | 86 | | | | | | | |
| lymphoid | 64 | 40 | | | | | | | | | | | | | | | |

- From the above map we extract the below six candidate signatures that ***commonly*** occur in tumors of the bladder and tumors in other organs.[5]




***Do these signatures arise in the healthy bladder epithelium and if detectable, could they inform risk in remote organs?***

## Conclusion:

- The sequencing of urine cell pellets is feasible for the detection of mutational signatures, the etiology of which may hold significance beyond the bladder.
- Further investigation may inform ways in which 'shared' signatures detected in the bladder could inform risk and screening in a public health and early detection setting of other organs.
- Future questions below include,

***'what does the signature look like in healthy epithelium? 'do these signatures change over time?' – 'are these signatures present in the urine of patients who develop tumors in remote organs?'***

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**References:**


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