Error Corrected Sequencing for Clonal Expansion

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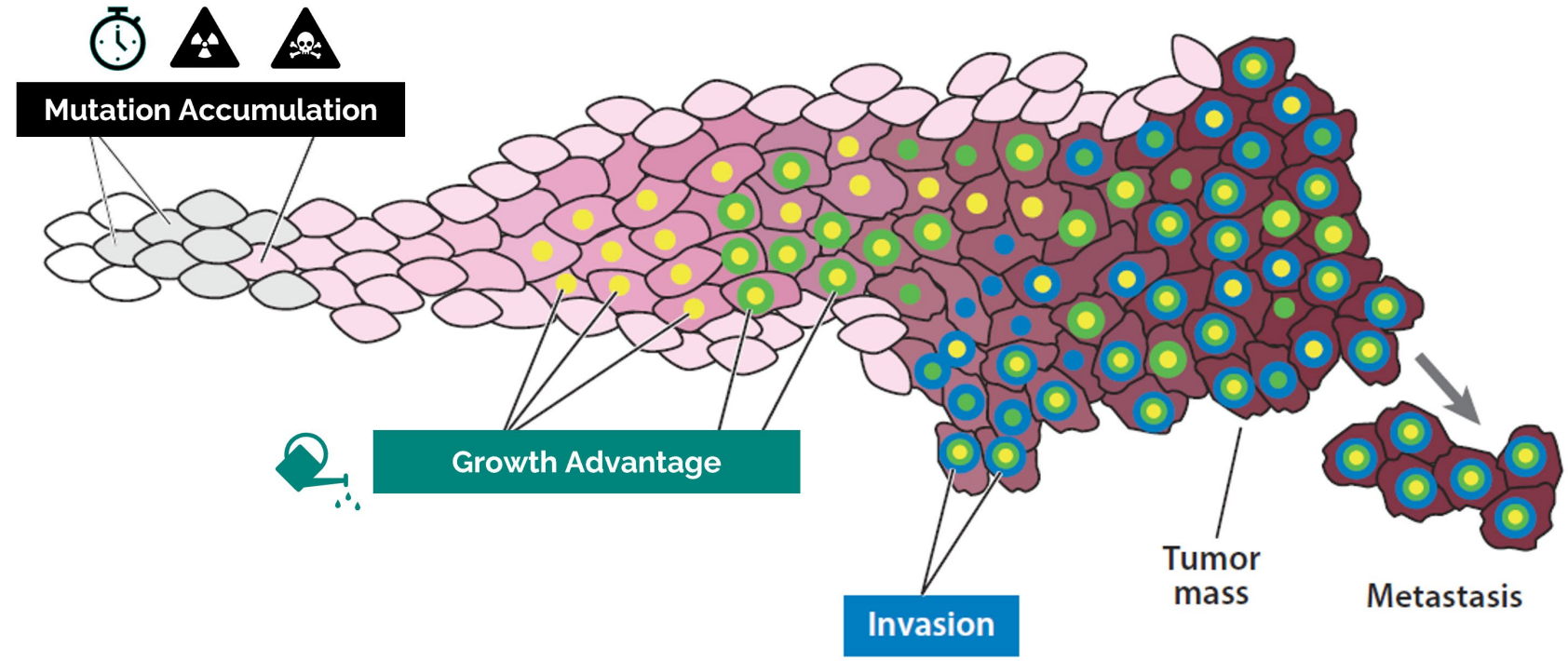
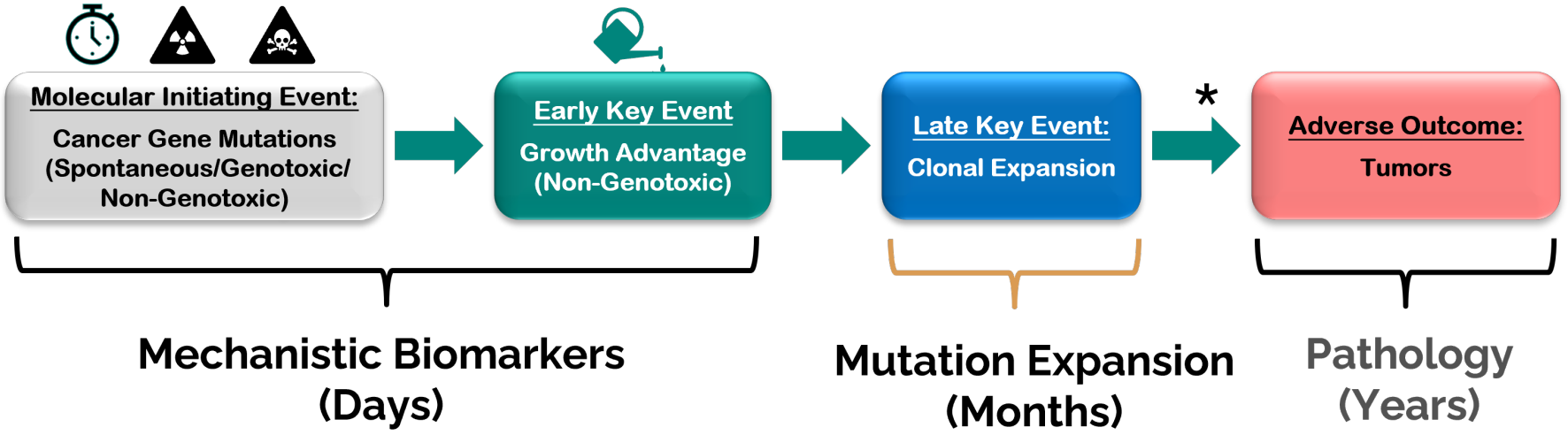
Backgrond

Progression of Cancer Biology: Opportunities for Earlier Detection





- Sensitive detection of early tumorigenesis (clonal expansion) could allow assessment of w/in months, prior to gross histopathology
- Combining MIE biomarkers with clonal expansion detection in short-term rodent studies could provide a strong weight-of-evidence alternative to 2yr carcinogenesis studies aligned with ICH S1B (R1) addendum

*Challenge - understand the thresholds from initiation, promotion to progression

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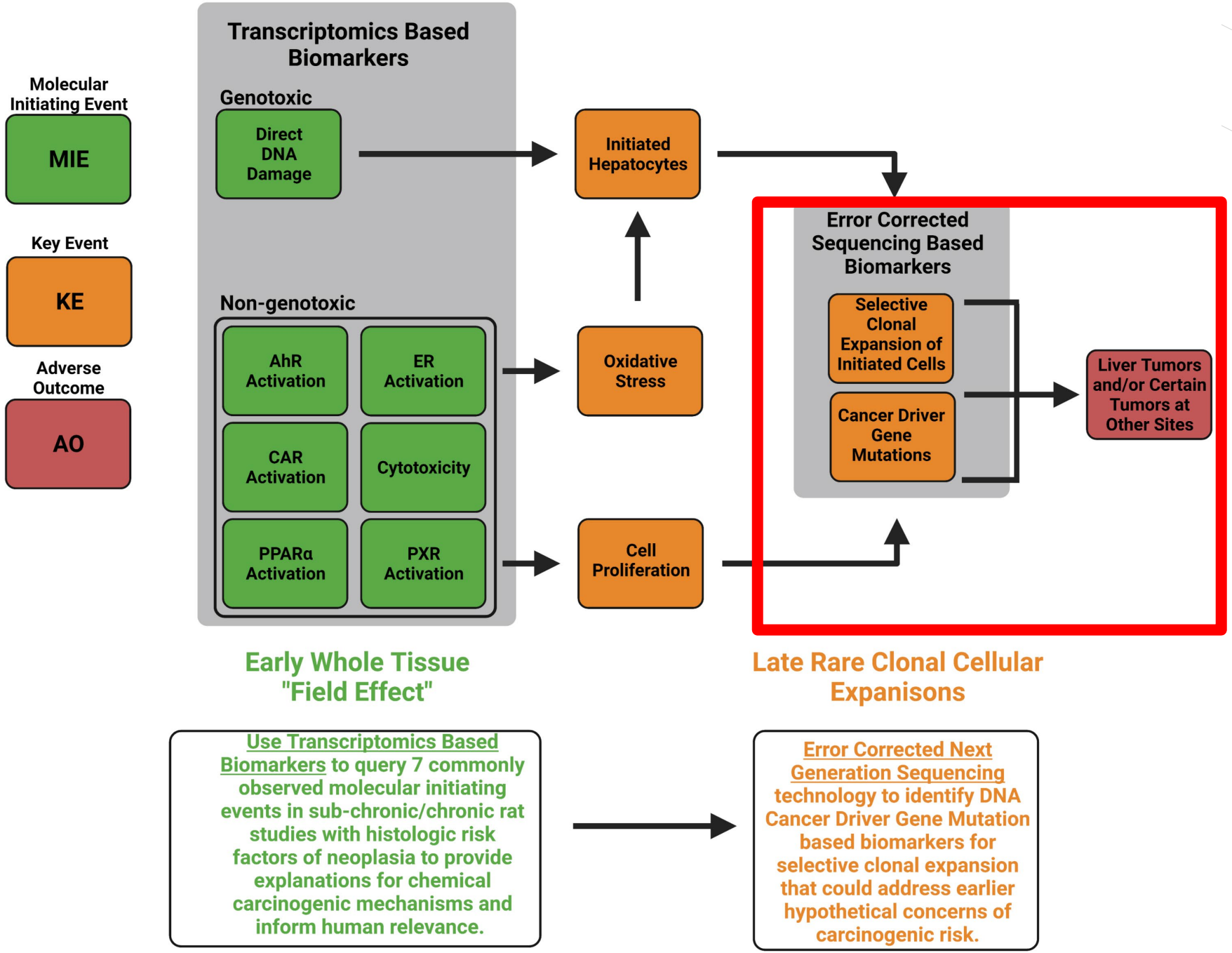

Corton et al 2022

Background

- Error Corrected Sequencing for clonal expansion can be applied in a weigh-of-evidence analysis to determine the carcinogenic potential of a test agent
- Gene expression biomarkers and clonal from short-term rat studies with test article contribute to WOE to waive the need for a 2-year bioassay
  - E.g., ICH S1B (R1) (Pharmaceuticals)

Method Description

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- Error corrected sequencing technologies that individually barcode double-stranded DNA molecules can dramatically reduce the technical error rates to detect rare mutations
  - Duplex sequencing technology have a mutagenesis panel of genes that can also be used to predict genotoxicity of a compound.
- Cancer driver gene mutation biomarkers can provide early molecular evidence of a chemical's potential tumorigenic risk when found to occur in cells of a tissue from treated animals.
- For short term in vivo rodent study tissue samples (3-6 months), DNA is extracted from tissues, error corrected sequence library prep kits are used to make cDNA, DNA is sequenced to detect mutations in a panel of cancer driver genes (v1)

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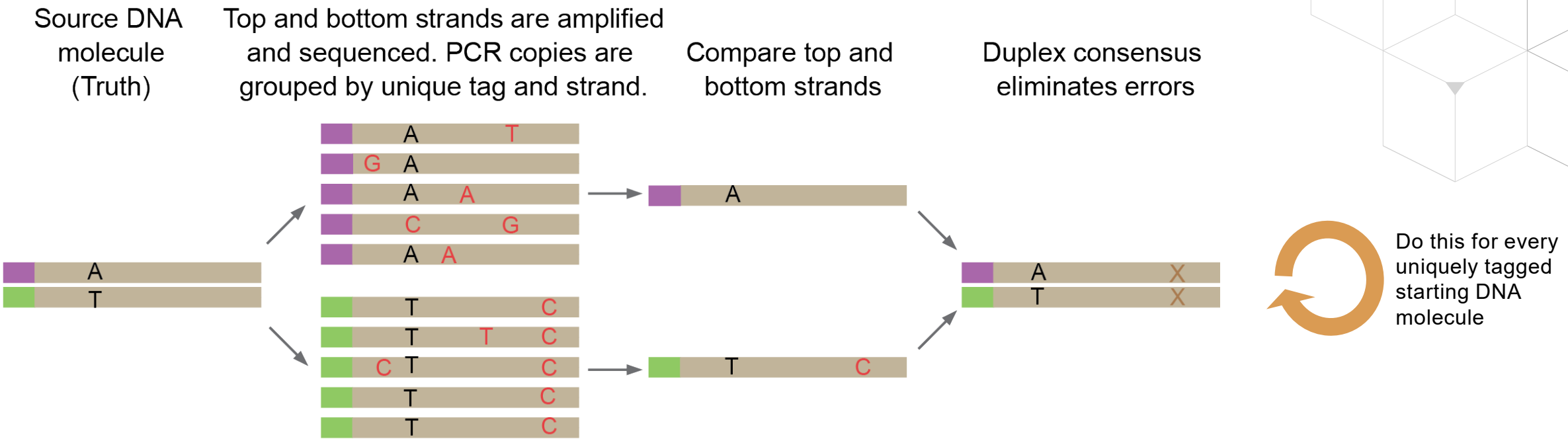
Duplex Sequencing Enables Quantification of Very Rare Mutations



- Duplex Sequencing overcomes error-based limitations of standard sequencing by independently tracking both strands of individual DNA molecules and comparing the results to eliminate errors in silico. Other error corrected sequencing methods can also be used
    - Errors are eliminated computationally by comparing results for the two barcoded strands after separate labeling, amplification and sequencing
- Reduces error rate from 1/100 to 1/10,000,000 base pairs

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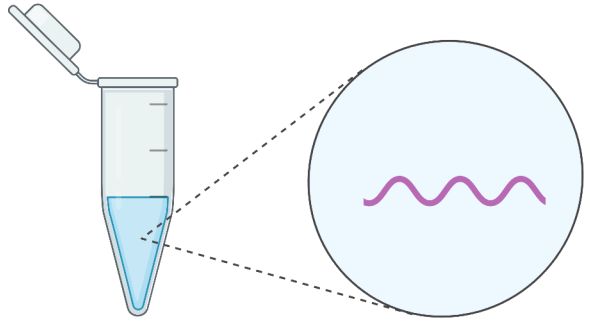
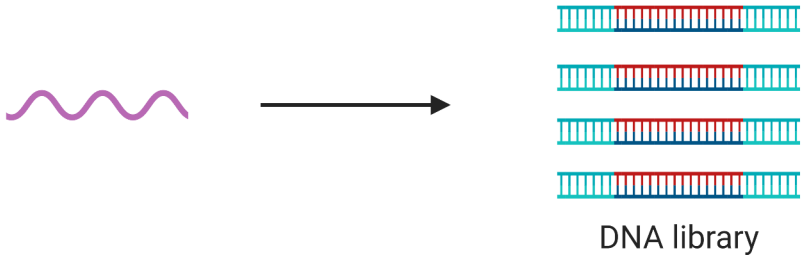
Sequencing Workflow

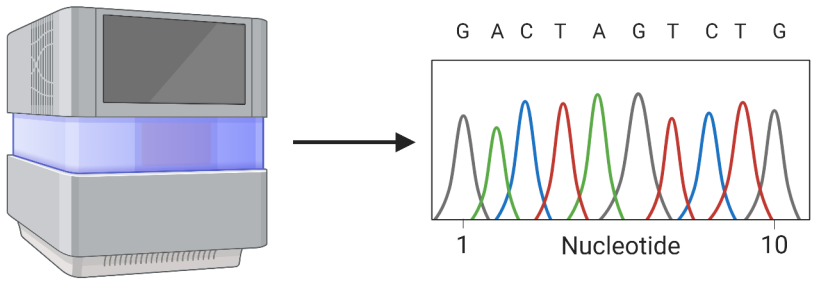
Step 1:
DNA extraction



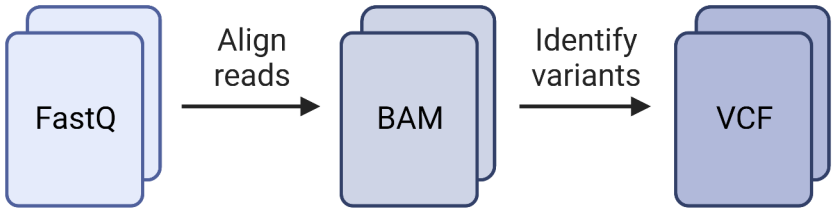
Step 2:
Library preparation



Step 3:
Sequencing



Step 4:
Analysis



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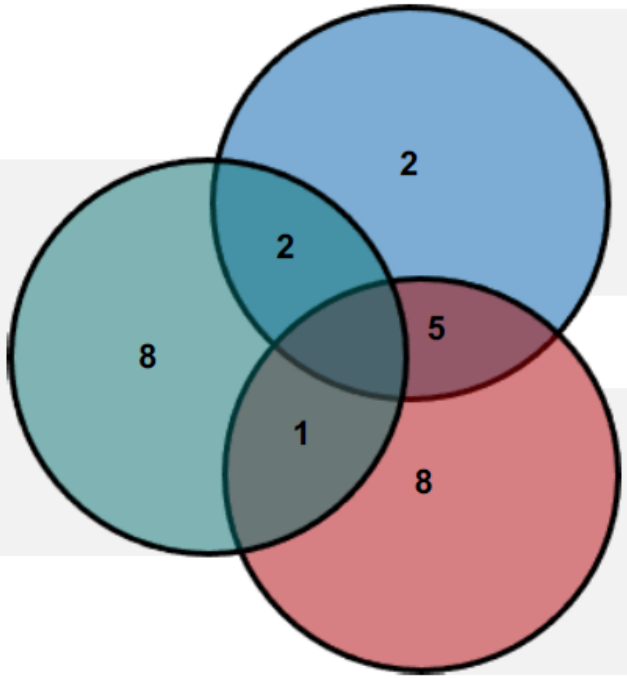
Rat carcinogenesis panel v1

Panel design covers 26 cancer driver genes that were identified in WT-rat tumors (rat), Riva *et al*. (mouse), and CarcSeq / COSMIC (human)

11 Rat Cancer Driver Genes (putative)
PRJ00067

Nfe2l2, Ctnnb1, AABR07025818.1, Kcnq 5, Atp5f1b, Atxn2,
Aldoa, Ggt1, Ghrhr, Rad51c, Sez6l2

Green text: Not known cancer driver gene



9 Mouse Cancer Driver Genes
Riva et al., Nat Genetics (2020)

Ctnnb1, AABR07051892.1 (Lrp1b), Cnot3, Egfr, Fgfr2, Kras, Hras, Braf, Tp53

14 Human Cancer Driver Genes
McKim et al., Tox Sci (2021), COSMIC

Pik3ca, Tp53, Stk11, Kras, Hras, Braf, Egfr, Nfe2l2, Apc, Setbp1, Tert, Arid1a, Arid2, Keap1

26 Cancer Rat Driver Gene Panel

Intervals: 315
Target footprint: 52,182 bp
Bait footprint: 93,116 bp

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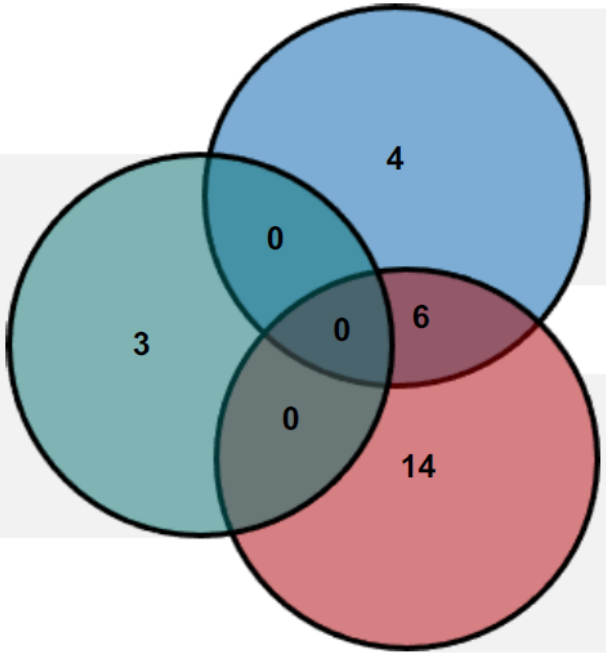
Mouse carcinogenesis panel v1

Panel design covers 27 cancer driver genes that were identified in Tg-rasH2 tumors, Riva *et al*. (WT-mouse), and CarcSeq / ICGC (human)

3 Tg-rasH2 Cancer Driver Genes (putative)
PRJ00183

Human HRAS, Cdh2,
Tsg101

Green text: Not known cancer driver gene



10 Mouse Cancer Driver Genes
Riva et al., Nat Genetics (2020)

Ctnnb1, Lrp1b, Cnot3, Egfr, Fgfr2, Kras, Hras, Braf, Trp53, Epha3

20 Human Cancer Driver Genes
Harris et al., Tox Sci (2021), ICGC

Pik3ca, Trp53, Stk11, Kras, Hras, Braf, Egfr, Lrp1b, Nfe2l2, Apc, Setbp1, Tert, Rb1, Axin1, Cdkn2a, Pten, Acvr2a, Foxl2, Kmt2c, Nras

27 Cancer Mouse Driver Gene Panel
Intervals: 220
Target footprint: 30,084 bp
Bait footprint: 63,324 bp

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Context of Use

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- We see this being useful as a tool in part of a weight of evidence for chemicals that require 2-year bioassays. Could use information from 3—6 month studies to provide information for 2-year studies
  - Also provide mechanistic information.
- Both genotoxic and non-genotoxic chemicals could be detected since we are looking at cancer driver gene mutations
- No regulatory submissions to date

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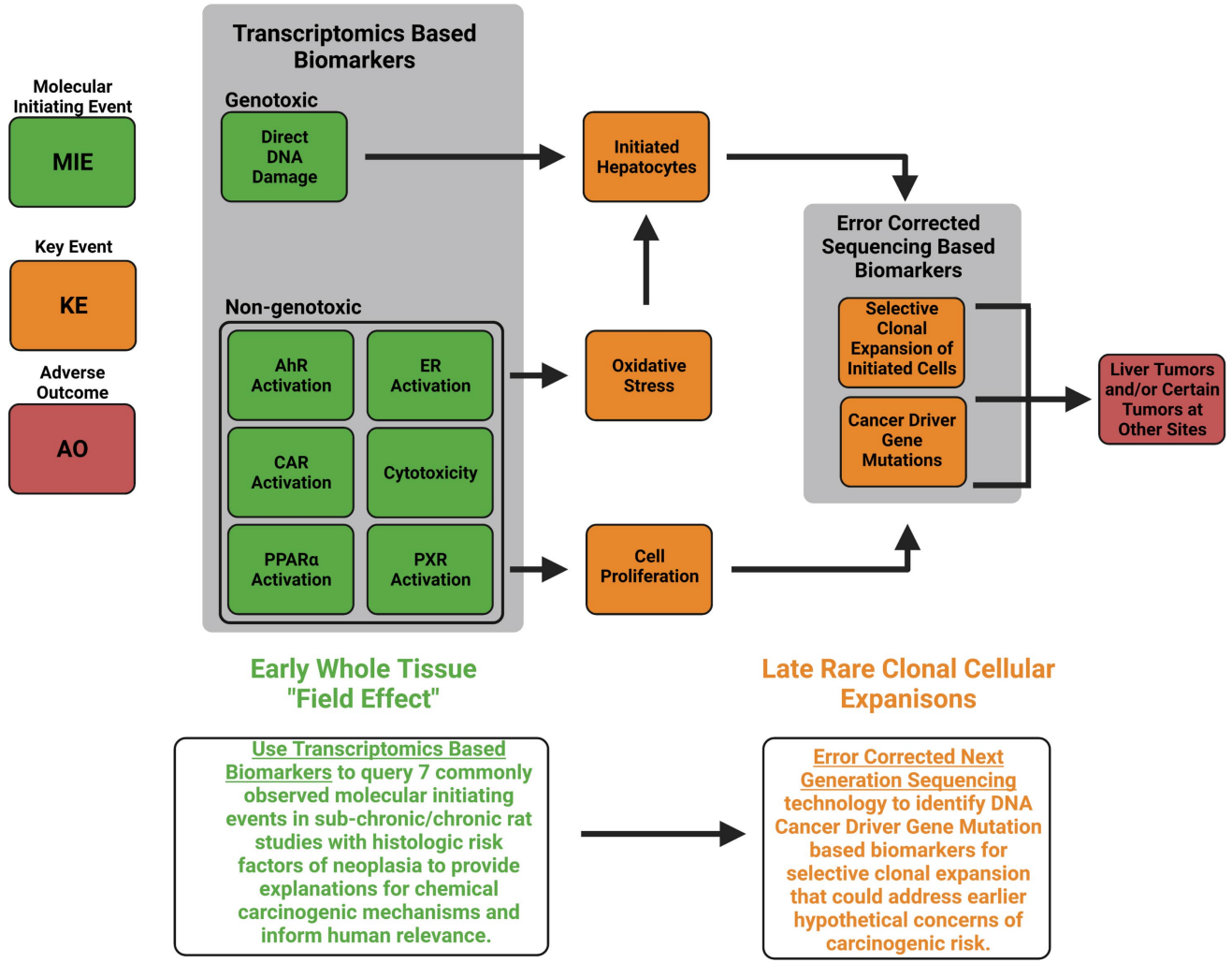
3Rs

- **Reduction:** using less animals by decreasing the need for the 2-year bioassay
- **Refinement:**
  - shorter tests and more mechanistic information.
  - Using existing 3-6 month studies
- Long term goal would be a **replacement** of 2-year bioassay

Biological Relevance

Mechanistic understanding: How does the information provided by your method support known mechanistic knowledge of the carcinogenesis process (e.g., an AOP or toxicologically relevant biological process)?



Reference compounds: What are well-characterized and understood compounds that can be used or were used to assess the scientific validity or transferability of your method?

- Well characterized non-genotoxic carcinogens needed to develop technology
- HESI work has identified non-genotoxic carcinogens
  - We also have an inventory of pre-existing samples to use
- In future, recommend use 2 compounds for lab capabilities

Comparison to existing laboratory animal methods:

- This is a refinement but shortening the time needed from 2-years to 3-6 months and providing mechanistic information.
- Other tools, like information on molecular iniating events, would be needed to understand human relevance and safety.
- Relevant to human biology – measuring changes in endogenous mammalian genes that are also cancer driver genes in humans

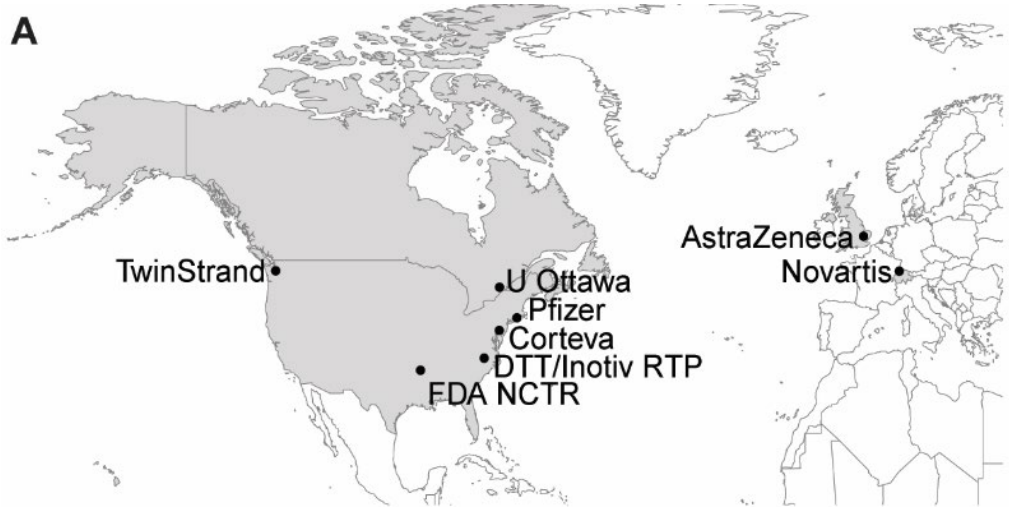
Technical Characterization

Technical Characterization

B. How has robustness (i.e., the ability of the method to be reproduced under different conditions or circumstances, without the occurrence of unexpected differences in the obtained results) been evaluated?

- Error corrected sequencing has been done in multiple labs for the **mutagenesis** panel for genotoxicity, specifically for DNA extraction and library preps. For carcinogenesis, only two labs.

Multi-site Experimental Study for Duplex Sequencing for Mutagenesis Panel



Zhang et al in prep.

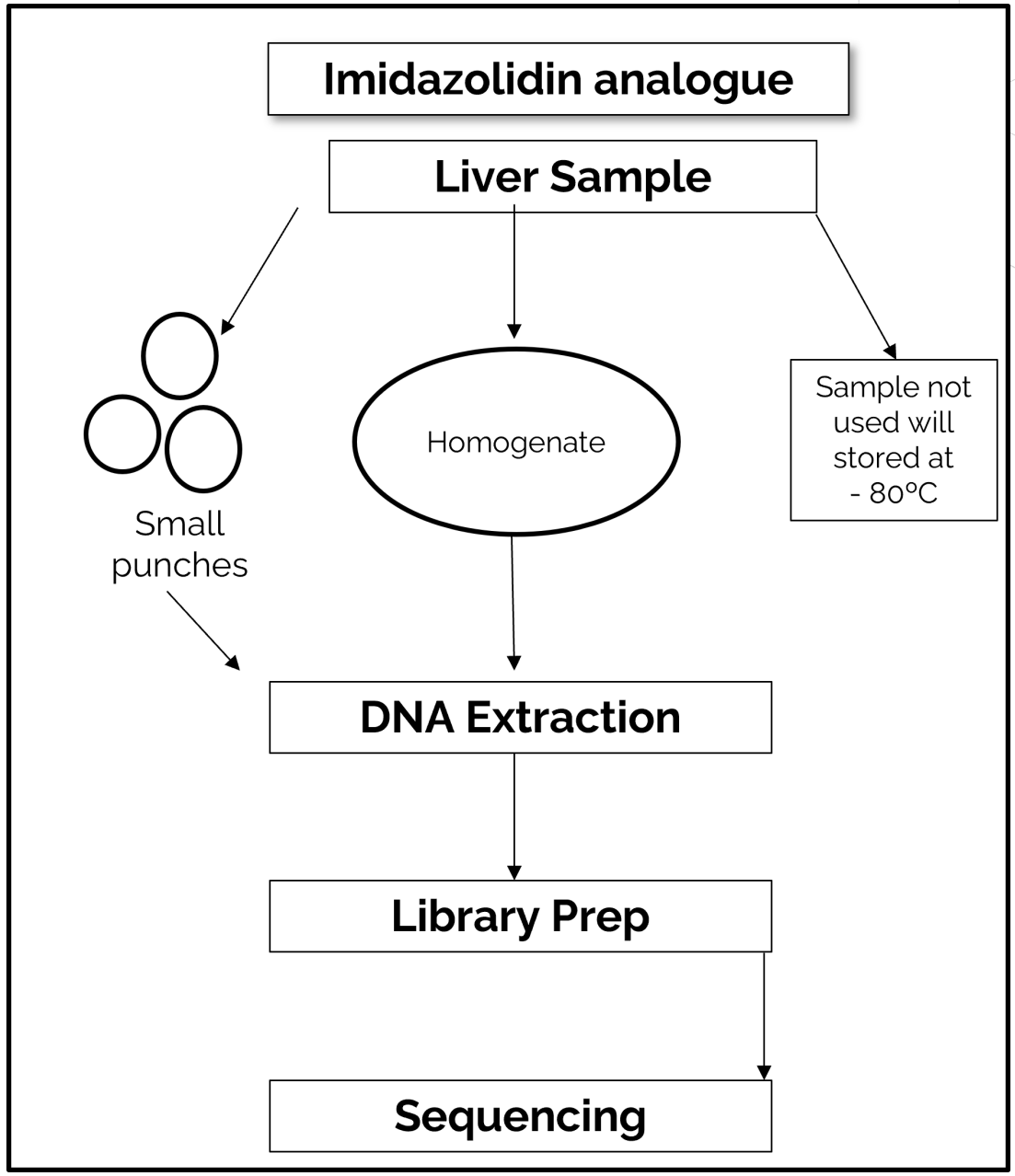
- This study supports the use of error corrected sequencing (Duplex Sequencing workflow) in multiple labs to show sensitivity, specificity, accuracy, precision, and reproducibility.

Multi-site Experimental Study for Duplex Sequencing for Mutagenesis Panel

Current Steps

Current Pilot – Study Design

Determine if there is a difference in the clonal expansion response between treated and control samples for **punches versus homogenates** of samples



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Further Development of ECS for clonal expansion

1. **Additional Compounds/Studies:**
   - Studies with additional compounds will help establish sensitivity/specificity of the approach as well as the ability to distinguish tumorigenic from non-tumorigenic dose levels

2. **Additional Genes:**
   - A sensitive assay will require broad coverage of potential driver genes across different NGT mechanisms
   - Studies with additional compounds will help identify gaps in existing panels and WES on these samples can be used to identify novel driver genes.

3. **Establish Thresholds:**
   - Establish normal baseline of expanded clones across time points, and normalized thresholds of concern to enable early risk assessment of novel compounds

4. **Regulatory Acceptance:**
   - Ultimate goal is to use as part of a weight-of-evidence approach for carcinogenic risk assessment and potential waivers of long-term carcinogenesis studies

Closing/Contact

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