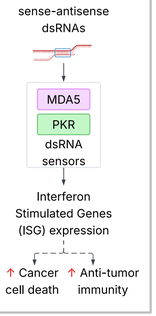
# Identifying actionable dsRNA biomarkers from sense-antisense transcript pairs

Otto Morris, Laura M. Richards, Jenny Rudnick, Kelly M. Biette and Aurora S. Blucher; *Recursion*

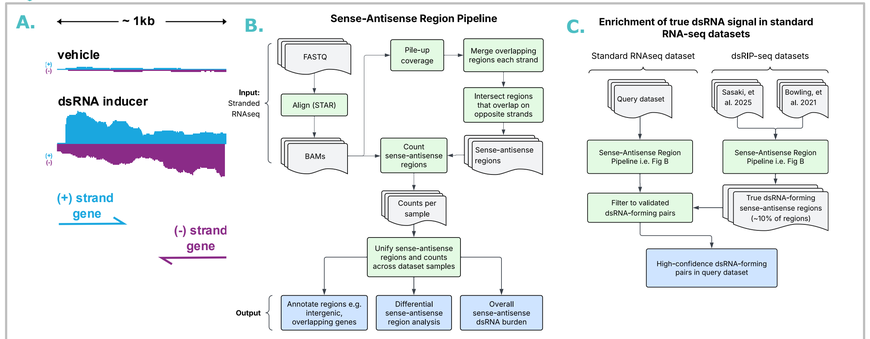
## 1 INTRODUCTION

Endogenous double-stranded RNA (dsRNA) accumulation in tumors is a rapidly emerging area of interest for therapeutic targeting and precision biomarker development. High dsRNA levels activate innate immune sensors such as PKR and MDA5, triggering interferon responses that induce immunogenic cancer cell death. The field needs predictive biomarkers that measure dsRNA directly. Current approaches instead rely on downstream interferon gene expression, which is time-delayed and confounded by feedback loops when the drug target is itself an interferon-stimulated gene. Standard transcriptomic analyses cannot fill this gap because they are blind to dsRNA.

We developed a computational pipeline that quantifies dsRNA directly from standard stranded RNA-seq data by focusing on sense-antisense transcript pairs: independently transcribed RNAs from overlapping genomic loci that hybridize to form dsRNA. This yields a proximal, dose-dependent biomarker of direct target engagement for RNA-regulating enzymes, upstream of interferon. Scoring these conserved pairs across patient tumors identifies cohorts with elevated baseline dsRNA most likely to respond to dsRNA-inducing therapies.




## 2 METHODS




**A. Targeted compounds strongly upregulate sense-antisense dsRNA**. Sense-antisense pairs form when transcripts from opposite strands of the same genomic locus hybridize. Baseline dsRNA levels remain low in untreated samples, but treatment with an dsRNA inducer drives a robust increase in read coverage across both strands of these paired regions.

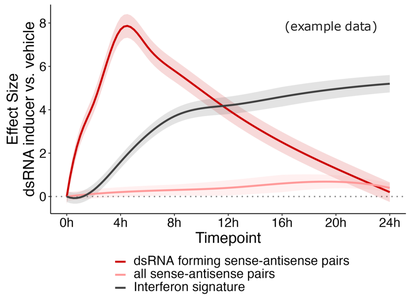
**B. Coverage-based inference enables bespoke sense-antisense dsRNA quantification**. A computational pipeline defines sense-antisense regions by detecting overlapping read coverage on opposite strands. This approach identifies sense-antisense regions within individual samples, enabling downstream genomic annotation, differential expression analysis, and overall dsRNA burden assessment.

**C. dsRIP-seq integration isolates true dsRNA duplexes.** Standard RNA-seq detects many overlapping regions that do not actually form stable dsRNA. Running the pipeline first on dsRIP-seq data—where a dsRNA-binding antibody specifically enriches dsRNA—identifies bona fide dsRNA-forming duplexes. Applying this filtered dataset to a second run of the pipeline on standard RNA-seq data eliminates non-hybridizing pairs, yielding the high-confidence dsRNA regions essential for accurate downstream analysis.

## 3 RESULTS

### Target Engagement Biomarker

**A. dsRNA kinetics track direct target engagement**




- Treating a xenograft cancer model with a dsRNA inducer drives rapid sense-antisense dsRNA accumulation within 1h. Tracking drug clearance kinetics, dsRNA levels return to baseline by 24 hours.
- Filtering for true duplexes using dsRIP-seq is essential. Indiscriminate analysis of all overlapping transcripts washes out the target engagement signal.
- The downstream interferon response is delayed, consistent with a transcriptional readout. Interferon signaling rises significantly at 8h and remains high at 24h, confirming immune sensor activation follows dsRNA accumulation.

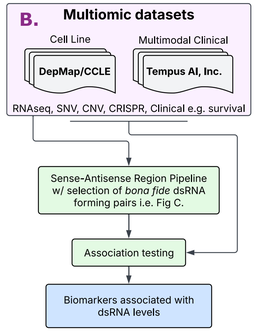
### Predictive Biomarker

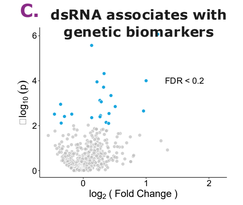
**B. dsRNA scoring scales to multiomic cohorts:** To identify biomarkers stratifying patients likely to benefit from dsRNA-inducing therapies, we scored sense-antisense dsRNA across DepMap (cell line) and Tempus (multimodal clinical) datasets.

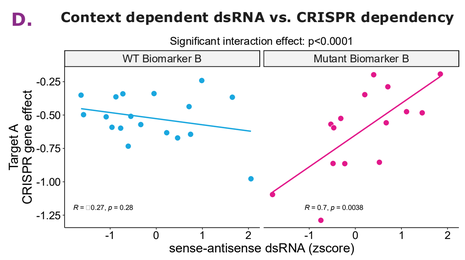
**C. dsRNA maps to clinically tractable genetic biomarkers:** An RNA-seq-based dsRNA score is hard to deploy clinically. In a cohort of 1,300 NSCLC patients from the Tempus dataset, mutations and copy number alterations are significantly associated with baseline dsRNA, offering actionable surrogates for dsRNA status.

**D. dsRNA predicts context-dependent CRISPR dependencies:** In a subset of DepMap cell lines with stranded RNA-seq, Target A dependency (more negative gene effect) strengthens only in low-dsRNA lines carrying a Biomarker B mutation, showing Biomarker B reshapes how dsRNA relates to dependency.

**E. dsRNA stratifies clinical survival, again gated by Biomarker B:** In clinical samples, high baseline dsRNA is associated with improved survival, but only in Biomarker B wild-type samples; the association is lost in Biomarker B mutants. dsRNA cannot be read in isolation; its value as a biomarker is gated by genetic context, with direct implications for patient selection.



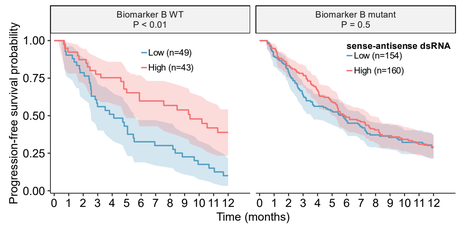




**Figure Legend: A.** Time-course of sense-antisense dsRNA accumulation in a xenograft model treated with a dsRNA inducer. **B.** Schematic of the pipeline testing dsRNA associations in DepMap and Tempus datasets **C.** Pairwise associations (Wilcoxon rank-sum) between SNV/CNV status and baseline dsRNA scores in the Tempus NSCLC cohort (RS.v2 and xT assays). **D.** Target A DepMap dependency versus dsRNA scores. Linear modeling reveals a significant interaction: Target A dependency correlates with dsRNA only in Biomarker B mutant lines. **E.** Kaplan-Meier survival curves for the Tempus NSCLC cohort, stratified by baseline dsRNA score and Biomarker B mutation status.

## 4 DISCUSSION AND CONCLUSIONS

Raising dsRNA turns a tumor's own transcripts into a trigger for innate immune sensing, driving immunogenic cancer cell death. These therapies are usually tracked through the interferon response they induce, but that readout lags behind the drug and becomes circular when the target is itself an interferon-stimulated gene. The more direct measurement is already sitting in routine data: scoring sense-antisense transcript pairs reads dsRNA straight from standard stranded RNA-seq.

- **A proximal readout of target engagement.** The dsRNA signal rises within an hour and scales with dose, reporting whether a drug reached its target well before the interferon cascade would.
- **dsRNA is only meaningful in genetic context.** High baseline dsRNA marks tumors primed for immune activation, but the same score carries different weight across genetic backgrounds. Interpreted with that context, it stratifies patients for dsRNA-inducing therapies and immunotherapy combinations.
- **A tractable path to patient selection**. A bespoke per-patient RNA-seq score is hard to deploy, however, the score maps onto recurrent mutations and copy number changes, providing genetic surrogates that fit standard molecular panels.

## 5 ACKNOWLEDGEMENTS AND REFERENCES

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1. Morris, O. (2026). Generating dsRNA complementary transcript regions for biological response prediction and perturbation mapping using sequencing models (U.S. Patent Application Docket 19/451,599)
2. Sasaki, N. et al. Mol Cell 85, 770–786.e7 (2025).
3. Bowling, E. A. et al. Cell 184, 384–403.e21 (2021).
4. Ghandi, M. et al. Nature 569, 503–508 (2019).

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