Dysregulation of the RNA modification m^{6}A promotes cancer progression and provides therapeutic opportunities

Abstract #6815

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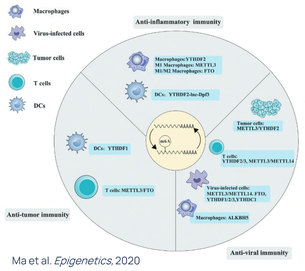
Background on RNA Modifications

More than 50 RNA modifications (mods) have been reported in humans, 170 across all kingdoms of life. **m^{6}A, inosine and pseudouridine** are the most frequent and well studied RNA mods in mRNA. RNA mods are implicated in all aspects of RNA biology including **splicing, structure, lifetime, trafficking and translation** and are dysregulated in human **cancers, cardiovascular and neurological diseases**.



Ma et al. *Epigenetics*, 2020

m^{6}A

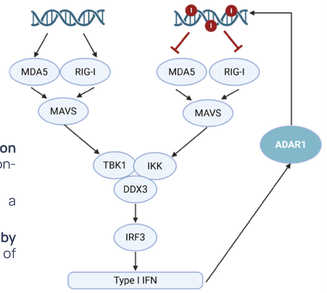
m^{6}A is installed co-transcriptionally and is dynamically regulated by METTL3, FTO, and ALKBH5.

- **m^{6}A regulates innate and adaptive immunity**, shaping immune cell, activation, differentiation, and inflammation
- **m^{6}A modulates anti-tumor immunity and immunotherapy response**, highlighting its therapeutic potential
- m^{6}A machinery (writers, erasers, readers) regulate immune cell function across diverse cell types

Inosine

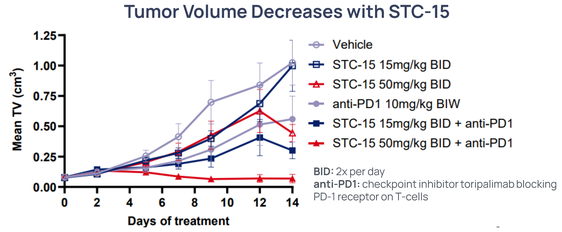
Adenosine to inosine **(A-to-I) editing is catalyzed post-transcriptionally by ADAR1 and ADAR2** in dsRNA regions.

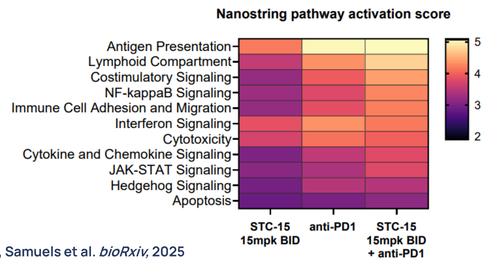
- **A-to-I editing can prevent the activation of innate immunity** and type I interferon-mediated responses.
- Abnormal ADAR editing is present in a variety of cancers.
- **Inosine is recognized as guanosine by cellular machinery**, leading to a variety of biological outcomes.



METTL3 Inhibitor STC-15 Reduces Tumor Volume

STC-15 is a METTL3 inhibitor developed by STORM Therapeutics and is currently in Phase 1b dose escalation.

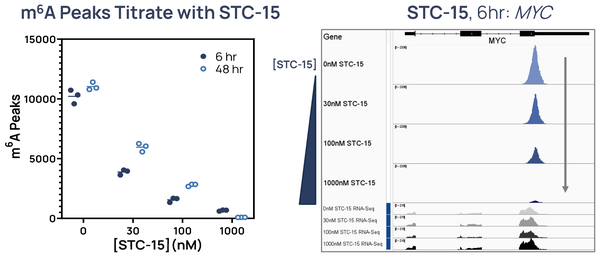




Fischl, Samuels et al. *bioRxiv*, 2025

Global and Gene Level Loss of m^{6}A Observed with METTL3 Inhibition

Study Overview: 1000 ng of total RNA from the CAOV3 ovarian cancer cell line treated with 0 to 1000 nM STC-15 at 6 and 48 hr was processed using the EpiPlex assay to profile m^{6}A and RNA-seq.



- Global loss of m^{6}A is observed at the peak level, where a gradual decrease in m^{6}A peaks at both time points is observed
- Loss of m^{6}A is recapitulated at the gene level, as shown for example gene *MYC*, where loss of m^{6}A abundance in the 3'UTR is observed.
- Dysregulation of m^{6}A alters RNA transcript stability and translation efficiency, impacting tumor suppressor and oncogene expression

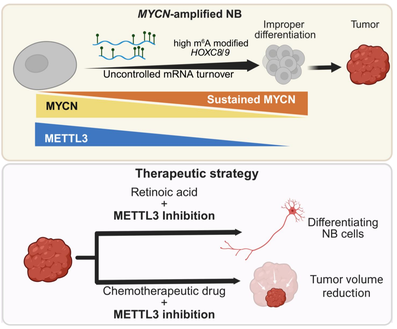
m^{6}A-Mediated Epitranscriptomic Regulation in High-Risk Neuroblastoma

Background:

- High-risk neuroblastoma (NB) is frequently driven by MYCN amplification and/or the alternative lengthening of telomeres (ALT)
- High-risk NB is therapy-resistant with poor outcomes
- Epitranscriptomic mechanisms may underlie tumor maintenance and treatment response

Previous Findings (Thombare, et al *EMBO J*, 2024; Vaid, et. al *NAR*, 2024):

- ALT+ NB depends on m^{6}A-modified lncRNA TERRA, with m^{6}A loss inducing telomere damage
- MYCN and m^{6}A cooperate to maintain an undifferentiated, tumorigenic state
- m^{6}A profiling showed m^{6}A-dependent regulation of NB-relevant transcripts
- METTL3 inhibition (STM2457) restores differentiation and reduces tumor burden



EpiPlex™ Detects RNA Modifications for Translational & Clinical Applications

HIGHLIGHTS

Multi-Modification Detection with One Workflow
Generates NGS libraries for **m6A, inosine, pseudouridine** and **RNA-seq.** The EpiPlex platform is modular and can readily scale to include other modifications.

Exceptionally Low RNA Input
Analyze tissue or blood biopsy samples with just 20 ng mRNA or 250 ng total RNA, or as low as 5 ng mRNA or 50 ng total RNA with higher duplicates.

Streamlined Workflow
Manually prepare up to 24 sequencing libraries in 7 hours. Automation friendly magnetic bead steps.

Quantitative Data
Spike-in controls allow for relative quantitation of RNA modifications between samples.

Cost Effective Sequencing
Recommended sequencing depth of 25M reads per library, 50M per sample (includes enriched & RNA-seq library)

Comprehensive Analysis Solution
Includes end-to-end analysis pipeline that employs machine learning powered peak caller optimized for RNA data.

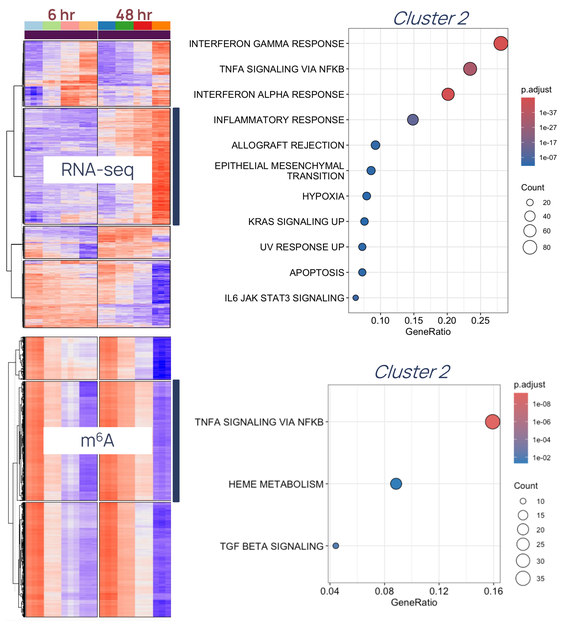
EpiPlex™ Reagent Kit

EpiScout™ Analysis Software

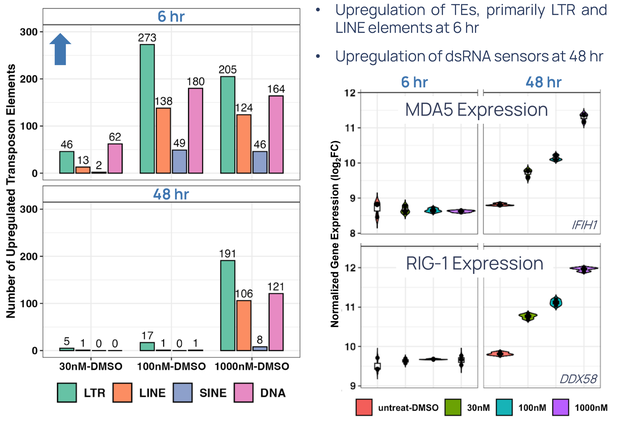
Currently available in 8- and 24-sample formats

Loss of m^{6}A Leads to dsRNA Accumulation and Upregulation of Immune Response

Clustering Analysis for m^{6}A and Gene Expression Enriches for Genes Involved in Immune Response

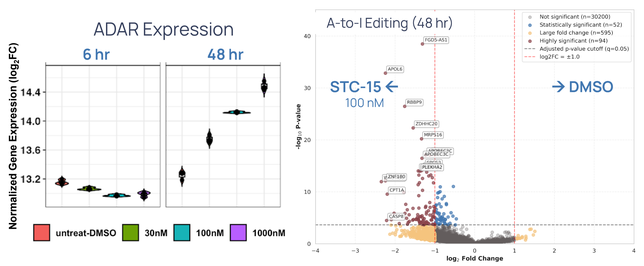


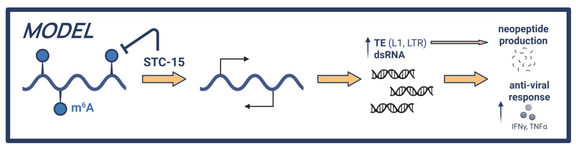
Transposable Elements (TEs) are and dsRNA Sensor Transcripts are Upregulated with STC-15 Treatment



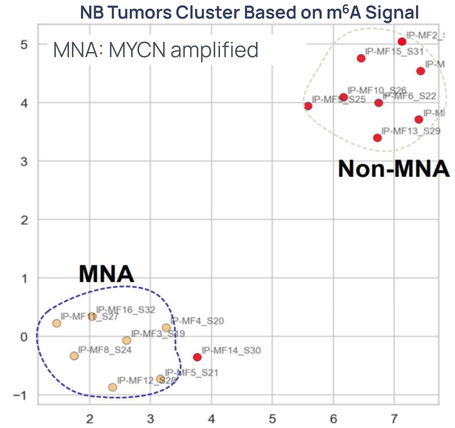
- Upregulation of TEs, primarily LTR and LINE elements at 6 hr
- Upregulation of dsRNA sensors at 48 hr

dsRNA Accumulation Supported by Increased A-to-I Editing





NB Tumors Cluster Based on m^{6}A Signal



clinical samples from 7 MNA amplified NB and 9 non-MNA NB

data generated from 300 ng FFPE total RNA using EpiPlex RNA mod encoding kit

SUMMARY

- m^{6}A represents a druggable regulatory axis in cancer with evidence across preclinical systems and clinical samples
- Integrated analysis of RNA modifications and gene expression can reveal underlying regulatory mechanisms that may inform biomarker discovery and provide therapeutic opportunities in cancer

Acknowledgements

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