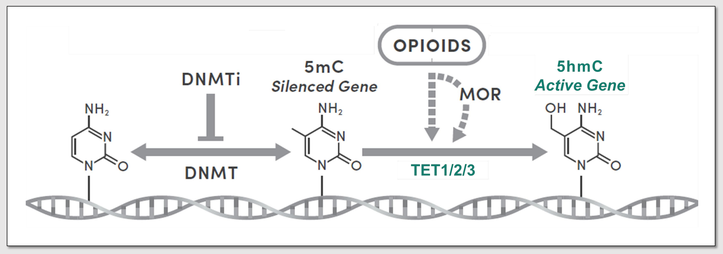
Potential Epigenetic Opioid-Chemotherapy Drug Interactions

C.L. Mathis, C.L. Bold, K.M. Watt, A.M. McKnite, J.E. Constance*
Division of Clinical Pharmacology, Department of Pediatrics, University of Utah

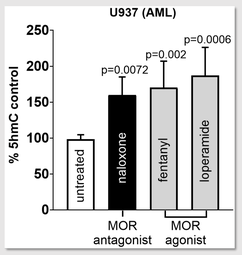
Introduction: Opioids Induce Changes in Cancer Cell Growth

- At therapeutic concentrations, opioids act both directly and indirectly (*via* the mu-opioid receptor (**MOR**)), on members of the ten-eleven translocase family (**TET1, 2, and 3**).[1]
- TET activity: Following DNA methyltransferase (**DNMT**)-mediated methylation, TET catalyzes the conversion of methyl cytosine (**5mC**) to hydroxymethylcytosine (**5hmC**): a process that initiates demethylation.[2]
- Opioid-induced changes in TET activity are poorly understood; however, leukemia pathogenicity is often characterized by dysregulated DNA methylation.
- DNMT inhibitors (**DMNTi**) are routinely used in the treatment of acute myeloid leukemia (**AML**): drug response is associated with tumor DNA 5hmC content as well as TET2 expression and activity.[3]
- Despite interactions with both MOR and TET, it remains unclear how and when opioids alter chemotherapy response.



Goal of Research: To assess opioid-induced changes to chemotherapeutic activity in leukemic cell lines.

Methods:



- 5hmC content increased in U937 cells + fentanyl, loperamide, or naloxone (72 hours, 1 μM), as per global DNA 5hmC ELISA (*right*):

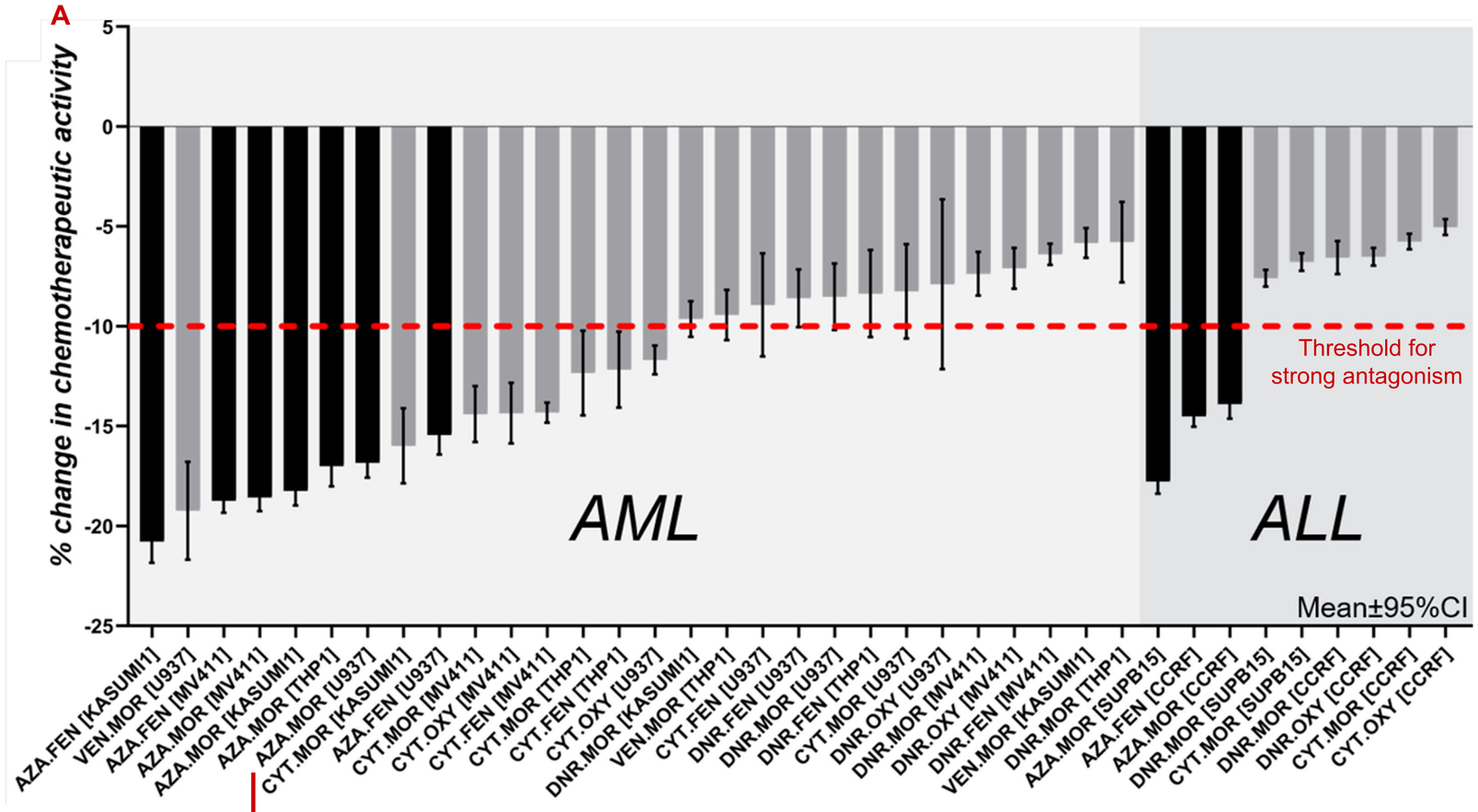
(A) Matrix Array for Cell Viability:
- Chemotherapeutic + Opioid in AML or acute lymphocytic leukemia (**ALL**) cell lines. **(B)** Azacitidine + Morphine in the AML cell line, U937 (*i.e.*, AZA.MOR[U937]).
- Synergy score is read as percent change in chemotherapeutic activity (SynergyFinder 3.0)[4]
- Black bars represent data for an opioid combined with the DNMTi, azacitidine.
- Grey bars represent data for an opioid combined with molecularly targeted and cytotoxic chemotherapeutics

(C) Influence of MOR on DNMTi activity:
- K562 cells + MOR agonist (morphine).
- One-way ANOVA, Tukey post-test.
- All data ≥4 independent experiments; mean ± 95%CI.

Abbreviations: azacitidine (**AZA**), venetoclax (**VEN**), cytarabine (**CYT**), daunorubicin (**DNR**), fentanyl (**FEN**), morphine (**MOR**), oxycodone (**OXY**), acute myeloid leukemia (**AML**), acute lymphocytic leukemia (**ALL**), **U937**: myeloid leukemia cell line, **K562**: myeloid leukemia cell line.

Approach: Multi-Sample, Multi-Drug Matrix Array of Cell Viability

A



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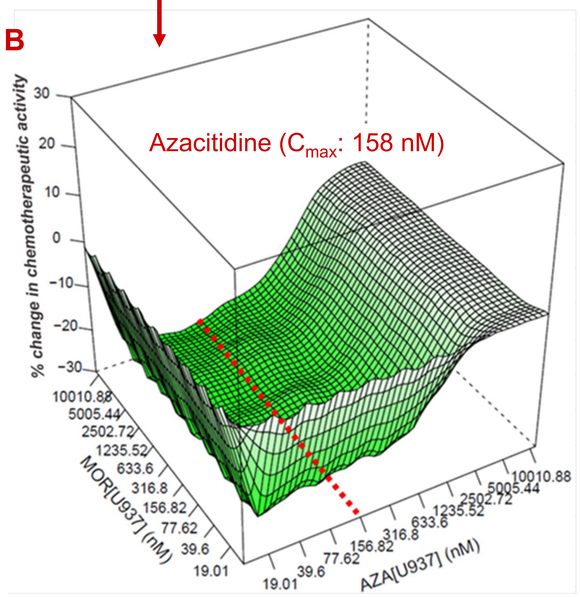

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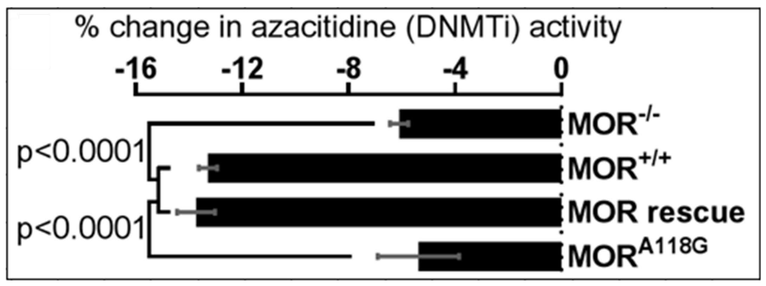

K562 MOR knockout cells ($MOR^{-/-}$), K562 wild type cells ($MOR^{+/+}$), K562 $MOR^{-/-}$ with stably re-introduced GFP-tagged *OPRM1* (MOR rescue), K562 $MOR^{-/-}$ with stably re-introduced GFP-tagged $OPRM1^{A118G}$ (MOR^{A118G}).[5]

- MOR function is affected by a common single nucleotide polymorphism in the *OPRM1* gene (A118G): 2% Afro-Americans, 8-30% Caucasians, and 50% of Asian populations.[6] MOR^{A118G} was less susceptible to opioid-induced chemoresistance to DNMTi.

Conclusions and Future Work:

- In diverse leukemic cell lines, analgesic opioids inhibited standard-of-care chemotherapy response.
- In the presence of analgesic opioids, MOR function exerts a suppressive effect on DNMTi.
- Profound inhibition for epigenetic targeted therapies was observed; further DNMTi was unable to overcome this inhibition.
- Subsequent work will focus on measuring relative 5mC and 5hmC modifications in cell lines +/- opioid treatment using nanopore sequencing.

References:

1.) *Cell Rep.* **2022**, *38*, 110253; 2.) *Nat. Rev. Genet*. **2017**, *18*, 517; 3.) *Leukemia* **2021**, *35*, 1873; 4.) *Nucleic Acids Res.* **2022**, *50*, W739; 5.) *J. Pain Symptom. Manage*. **2024**, *67*, 39 e35; 6.) *Anesthesiology* **2012**, *116*, 896.

Acknowledgements:

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