Macrophage mediated resistance to TKI therapy in ALK fusion positive non-small cell lung cancer

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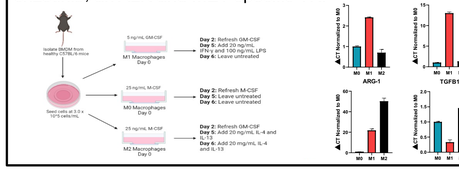
Background

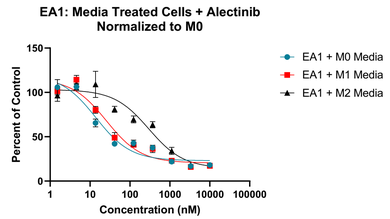
•Lung adenocarcinoma, a type of non-small cell lung cancer, comprises approximately 40% of all diagnosed lung cancers
•Around seven percent of lung adenocarcinoma cases bear an anaplastic lymphoma kinase (ALK) fusion oncogenic driver
•ALK+ disease is most often treated with a tyrosine kinase inhibitor (TKI), a targeted therapy
•Acquired resistance to targeted therapy is common but heterogenous across patients
•Signaling through epidermal growth factor receptor (EGFR) is a known mechanism of treatment resistance, but it does not account for the heterogeneity of resistance
•Previous analysis of patient tumors suggests composition of the tumor immune microenvironment (TIME) contributes to resistance to treatment, specifically CD14+ monocyte-derived macrophages
•When ALK+ human cell lines were exposed to CD14+ macrophages *in vitro*, TKI resistance increased by over 50-fold
•These results were confirmed *in vivo* with an immunocompromised mouse model
•We hypothesize that macrophages, specifically the M2 phenotype, contribute to TKI treatment resistance

Methods

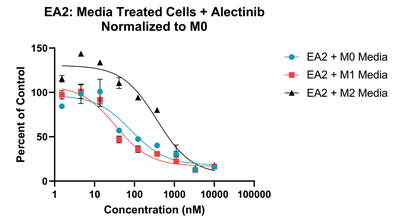
•Utilized EA-1, EA-2, and EA-3 ALK+ C57BL/6 murine cell lines derived at the University of Colorado
•Designed a protocol, depicted below, to polarize murine bone marrow-derived monocytes to M0, M1, and M2 phenotypes
•Confirmed polarization of monocytes to macrophages via qPCR, shown below
•Produced conditioned media by exposing each cell type to fresh media for 48 hrs and then filtering out the cells
•Utilized MTS, a colorimetric viability assay, to assess EA cell response to treatment when exposed to various conditioned media and treated with alectininb, a second generation ALK-specific TKI
•Repeated the MTS and treated the cells with either afatinib, an EGFR specific TKI, in conjunction with alectinib or crizotinib, an ALK and MET specific TKI



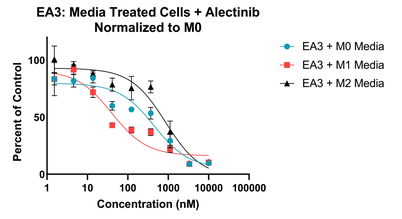
Results



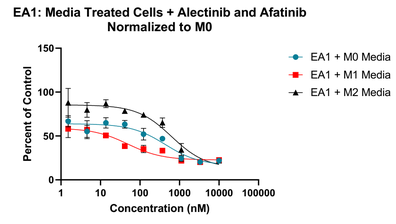
M0 Media IC50	M1 Media IC50	M2 Media IC50
14.40 nM	25.74 nM	297.50 nM



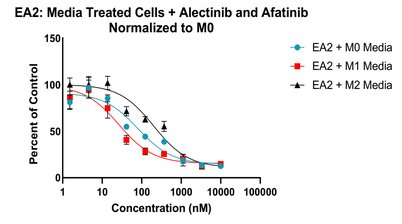
M0 Media IC50	M1 Media IC50	M2 Media IC50
81.34 nM	33.79 nM	366.10 nM



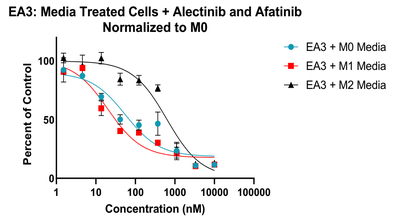
M0 Media IC50	M1 Media IC50	M2 Media IC50
452.30 nM	40.17 nM	871.10 nM



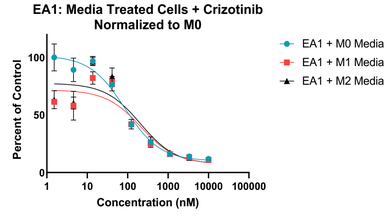
M0 Media IC50	M1 Media IC50	M2 Media IC50
440.10 nM	49.23 nM	629.80 nM



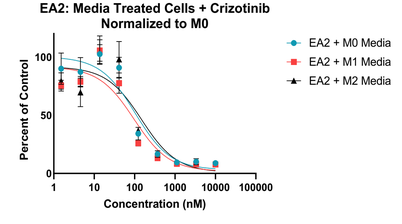
M0 Media IC50	M1 Media IC50	M2 Media IC50
80.10 nM	26.49 nM	202.40 nM



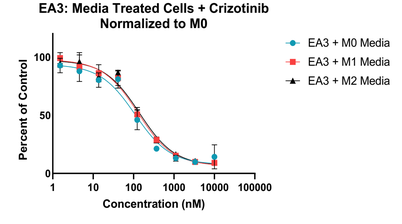
M0 Media IC50	M1 Media IC50	M2 Media IC50
55.32 nM	20.03 nM	625.20 nM



M0 Media IC50	M1 Media IC50	M2 Media IC50
85.30 nM	150.60 nM	207.5 nM



M0 Media IC50	M1 Media IC50	M2 Media IC50
104.0 nM	96.53 nM	148.70 nM



M0 Media IC50	M1 Media IC50	M2 Media IC50
109.30 nM	128.10 nM	139.90 nM

Conclusions

•Exposure to the signaling molecules associated with M2 macrophages leads to alectinib treatment resistance *in vitro*
•Treatment with alectinib and afatinib, ALK and EGFR specific TKIs respectively, did not alter levels of treatment response
•Treatment with crizotinib, an ALK and MET inhibitor, eliminated the resistance associated with exposure to M2 macrophage conditioned media
•A ligand associated with MET is likely partially responsible for observed resistance
•Further research is needed to understand the mechanism behind the M2 macrophage-mediated treatment resistance and the relationship between M2 macrophages and MET signaling in ALK+ lung adenocarcinoma

Implications

•Further identification of the mechanism behind M2 macrophage-mediated treatment resistance may lead to the development of M2 macrophage inhibitors
•This would allow for the extension of progression-free survival in patients with ALK+ lung adenocarcinoma and may have applications in other cancers

Acknowledgements

Schenk Lab
Erin Schenk, MD, PhD
Katherine Priest
Gregory Reis
Amanuail Gebregzabheir

University of Colorado Anschutz
Tissue Biorepository, Thoracic Oncology Initiative, and HIMSR

Other Contributors
Raphael Nemenoff, PhD
Amber Johnson, PhD
Emily Kleczko, PhD
Andre Navarro
Lynn Heasley, PhD
Trista Hinz
Dan Sisler

Disclosures

This research received funding from the National Institutes of Health, the LUNGevity Foundation, and the Cancer League of Colorado.

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