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

Patients with lung cancer bearing driver oncogenes such as epidermal growth factor receptor (*EGFR*) mutations or anaplastic lymphoma kinase (*ALK*) fusions often experience long term disease control on targeted therapy. Despite excellent initial responses, these tumors develop resistance to targeted therapy. While several mechanisms of targeted therapy resistance are known, they do not account for all cases of resistance. Based on our previous observations, we hypothesized tumor microenvironment resident monocytes and macrophages contribute to the development of resistance. We generated bone marrow derived macrophages from C57BL/6 mice and polarized them to an M0, M1, and M2 phenotype and harvested conditioned media from each. EA-1, EA-2, and EA-3 murine lung adenocarcinoma-derived cell lines bearing an oncogenic *ALK* mutation were exposed to conditioned media prior to treatment with the *ALK* fusion specific tyrosine kinase inhibitor (TKI) alectinib. Culturing cells in M2-CM resulted in at least a 10-fold increase in alectinib resistance across all 3 cell lines compared to the cell lines cultured in M0- or M1-CM. We next tested for the activity of known, clinically relevant bypass signaling pathways as mediators of M2-CM protection. Aftatinib, an inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2) family of proteins, did not alter M2-CM protection in the presence of alectinib. M2-CM did not impart TKI resistance in the presence of crizotinib, which inhibits ALK fusion activity and MET receptor tyrosine kinase activity, suggesting bypass signaling through MET drives the M2-CM resistance. Collectively, our work demonstrates TKI therapy may be modulated by the presence of and interaction with specific immune cells within the tumor microenvironment. These data support exploring macrophage targeting therapies to improve TKI responsiveness.