

## RESEARCH WATCH

## Stem Cells

**Major finding:** RTK inhibitors induce glioblastoma stem cells (GSC) to transition to a slow-cycling persister state.

**Mechanism:** KDM6 removes repressive H3K27me3 to upregulate Notch and neurodevelopmental genes in persister GSCs.

**Impact:** The epigenetic mechanism underlying persister GSC establishment may be targetable in glioblastoma.

## REVERSIBLE EPIGENETIC CHANGES CREATE REFRACTORY GLIOBLASTOMA STEM CELLS

Despite frequent alterations in receptor tyrosine kinases (RTK), RTK inhibitors have limited clinical efficacy in patients with glioblastoma, likely due to a subpopulation of refractory slow-cycling glioblastoma stem-like cells (GSC). Liao, Sievers, and colleagues investigated the molecular mechanisms underlying the therapeutic resistance of GSCs. In GSC cell lines, treatment with the RTK inhibitor dasatinib rapidly enriched a drug-tolerant, slow-cycling GSC “persister” population without evidence of genetic alterations that would confer resistance. When dasatinib was removed the cells reverted to a proliferative state, suggesting a reversible epigenetic mechanism of resistance. Compared with naïve GSCs, the persister GSCs exhibited upregulation of genes involved in primitive neurodevelopment and quiescence, consistent with a slow-cycling state, and increased Notch signaling. Small molecule-mediated inhibition of Notch activation reduced the growth of persister GSCs but had little effect on naïve GSCs, whereas overexpression of the Notch1 intracellular domain (N1ICD) induced a reversible growth reduction in the naïve GSCs, suggesting that Notch signaling promotes the switch to a slow-cycling, RTK inhibitor-resistant persister GSC phenotype. These findings were validated in primary glioblastoma tumors

which harbored Notch-positive cells with low expression of proliferation markers, indicative of persister GSCs. Chromatin immunoprecipitation sequencing showed that N1ICD and its cofactor RBPJ were enriched at the enhancers of neurodevelopmental genes in persister GSCs compared with naïve GSCs. Further, N1ICD-associated loci lost H3K27me3, a repressive chromatin mark, and gained H3K27ac as they transitioned from the naïve state to the persister state. This transition was accompanied by downregulation of the H3K27 methyltransferase EZH2 and upregulation of the H3K27 demethylases KDM6A and KDM6B, and, accordingly, persister GSCs were dependent on KDM6 activity. Collectively, these results indicate that a KDM6-dependent chromatin remodeling allows a reversible transition to a drug-tolerant, slow-cycling persister GSC state. Thus, targeting epigenetic and developmental pathways may target GSCs to potentially prevent drug resistance in patients with glioblastoma. ■

*Liao BB, Sievers C, Donohue LK, Gillespie SM, Flavahan WA, Miller TE, et al. Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. Cell Stem Cell 2016 Dec 15 [Epub ahead of print].*

## Clinical Trials

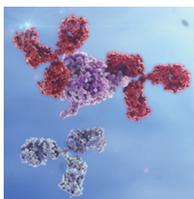
**Major finding:** First-line therapy with nivolumab plus ipilimumab has antitumor activity especially in PD-L1<sup>+</sup> tumors.

**Concept:** In an open-label phase I trial, adverse events were consistent with expected events from monotherapy.

**Impact:** Combined PD-L1 and CTLA-4 blockade may be effective as first-line therapy in patients with NSCLC.

## NIVOLUMAB PLUS IPILIMUMAB IS WELL TOLERATED AND ACTIVE IN NSCLC

First-line platinum-based combination chemotherapy achieves responses in approximately 30% of patients with advanced non-small cell lung cancer (NSCLC) without targetable mutations, but moderate-to-severe toxicity is observed and responses are not typically durable. The anti-PD-1 antibody nivolumab has been shown to improve survival compared with chemotherapy in patients with previously treated advanced NSCLC, and combined treatment with the anti-CTLA-4 antibody ipilimumab has improved activity compared with nivolumab or ipilimumab alone in melanoma, prompting Hellmann and colleagues to evaluate nivolumab plus ipilimumab as first-line therapy in an open-label phase I trial in patients with advanced chemotherapy-naïve NSCLC. Overall, 77 patients were treated: 38 received nivolumab every 2 weeks plus ipilimumab every 12 weeks, and 39 received nivolumab every 2 weeks plus ipilimumab every 6 weeks. The primary outcome was frequency of adverse events and serious adverse events, and secondary outcomes included objective responses and progression-free survival. Partial responses were achieved in 18 of 38 (47%) patients in the every-12-weeks cohort and 15 of 40 (38%) of patients in the every-6-weeks cohort.



Progression-free survival at 24 weeks was 68% in the every-12-weeks cohort and 47% in the every-6-weeks cohort. Further, among patients with 1% or more of tumor cells exhibiting PD-L1 expression, 12 of 21 (57%) in the every-6-weeks cohort and 13 of 23 (57%) in the every-12-weeks cohort achieved partial responses. Combination therapy was generally well tolerated, and adverse events were consistent with expectations based on adverse events observed after monotherapy. Treatment-related serious adverse events occurred in 32% of patients in the every-12-weeks cohort and 28% of patients in the every-6-weeks cohort. These findings indicate that first-line nivolumab plus ipilimumab has antitumor activity in patients with NSCLC and suggest that this combination may have comparable efficacy to standard chemotherapy with less toxicity, altogether supporting further clinical investigation, especially to treat patients with tumors expressing PD-L1. ■

*Hellmann MD, Rizvi NA, Goldman JW, Gettinger SN, Borghaei H, Brahmer JR, et al. Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol 2016;18:31-41.*

# CANCER DISCOVERY

## Reversible Epigenetic Changes Create Refractory Glioblastoma Stem Cells

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