Playing the melanoma endgame

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Running Title: Salvage therapies in resistant melanoma

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Summary

Treatments for melanoma are of two main types: targeted therapies and immune checkpoint inhibitors. However, both are only effective in a subset of patients and are limited by acquired resistance. Here, the authors present the preclinical basis to broadly target different forms of therapy-resistant melanoma.
In this issue of *Clinical Cancer Research*, Zhang et al. (1) describe the use of telomere-directed agents as a salvage option to treat targeted therapy and immune checkpoint resistant melanoma. The field of testing treatment options/combinations for melanoma patients is at the forefront of cancer therapies. Targeted therapies including BRAF inhibitors in combination with MEK inhibitors are only effective in a subset of patients, approximately 50% with tumors expressing BRAF V600E/K mutations. The effects of these combinations can be short-lived. Recent trials show median progression-free survival of 12-14 months on BRAF/MEK inhibitor combinations with acquired resistance ensuing. Immune checkpoint inhibitors such as anti-PD-1 (nivolumab/Opdivo and pembrolizumab/Keytruda) give durable remissions but these agents are effective in 30-40% of patients. Their effects can be augmented with a second immune checkpoint inhibitor, anti-CTLA4/ipilimumab but the trade-off is typically higher toxicity. Thus, an issue of increasing importance is how to treat patients that are either intrinsically resistant to targeted and/or immune based therapies or develop acquired resistance to these agents. This concern is underscored by the evidence that once patients become resistant to BRAF inhibitors, they frequently have rapid disease progression and often don’t benefit from immunotherapy (2).

Zhang et al. propose the use of a nucleoside, 6-thio-2’-deoxyguanosine (6-thio-dG) known to cause telomere damage as a salvage therapy for targeted inhibitor/immune checkpoint-resistant disease (Figure 1). Telomerase adds nucleotide repeats to the telomeric ends of chromosomes to maintain genomic stability, and is a highly relevant target in melanoma due to the frequent mutation of the telomerase promoter (3). This study is based on previous encouraging results testing 6-thio-dG in colon and lung cancer cell lines. Mechanistically, direct telomerase inhibitors function effectively only when cancer cells have short telomeres and can no longer divide resulting in a lag time between application and effectiveness. In the case of 6-thio-dG, a precursor of telomerase substrate 6-thio-dGTP, the main limitation of telomerase inhibitors is now side-stepped and potentially more efficacious. The current study shows broad efficacy of 6-thio-dG in melanoma cells lines *in vitro* and *in vivo* through effects on apoptosis and induction of senescence. Interestingly, these effects are independent of TERT promoter mutation status. Based on the up-regulation of resistance markers such as AXL (4) and telomere-related gene sets, the authors also assess the effects of 6-thio-dG as a second-line treatment for melanomas that are resistant to targeted and immune checkpoint inhibitor therapies. Analysis of telomere transcription signatures in resistant human melanoma biopsy samples is an important bed-side to bench component of the study. Through the use of BRAF inhibitor/combination BRAF plus MEK inhibitor-resistant cell lines, PDX models from tumors that progressed on immune-based therapies and syngeneic melanoma models showed that 6-thio-dG inhibits growth and down-regulates the resistance biomarker, AXL. The authors present these data as the basis for 6-thio-dG being used as a salvage treatment option to control targeted and/or immune checkpoint inhibitor resistant disease.

The current study provides a strong pre-clinical basis for exploring 6-thio-dG and other molecules inducing telomere dysfunction in melanoma. For future translation, several points should be considered. First, although 6-thio-dG neither inhibited the growth of normal human skin cells nor caused evidence of liver toxicity in vivo, these were short term studies. A more thorough analysis may be warranted. For example, it would be important to determine the effect of 6-thio-dG on highly proliferative cells that rely on telomerase such as somatic stem cells. Second, given the importance of tumor-infiltrating lymphocytes (TIL) cells to the action of immune checkpoint inhibitors, the effects of 6-thio-dG on TILs as well as peripheral immune cells could be assessed. Third, AXL expression is associated with therapy resistance but it is one of several receptor tyrosine kinases that are adaptively regulated. AXL may serve as a biomarker for a sub-population of cells within the tumor and given the cellular heterogeneity of melanoma, assessment of receptor tyrosine kinase expression at the single cell level would
provide a more comprehensive analysis of actions of 6-thio-dG and how it affects discrete cell populations. AXL is also one of the transcripts within a signature for innate anti-PD1-1 resistance (IPRES) and it would be interesting to determine the extent to which other IPRES signature genes are altered by 6-thio-dG. The analysis of biomarkers leads into the next point, resistance. In many of the PDX studies, tumors treated with 6-thio-dG are progressing within the 14-day timeframe of the experiment. Previous studies have highlighted the pathways leading to resistance to telomerase deficiency (5). Given the abundant evidence of evasion routes that melanomas take to overcome BRAF and MEK inhibitors, it seems that a similar endgame may result when heavily treated melanoma patients are given telomere-directed agents. Finally, causing telomere dysfunction may be particularly useful in subsets of melanoma for which there are low frequencies of BRAF mutations and lack of a strong ultraviolet mutation signature. One subset is acral lentiginous melanomas that arise on the palms of hands and soles of feet. This subset of melanoma is associated with worse survival outcomes than its cutaneous counterpart and display a high rate of alterations (translocation, copy gains and promoter mutations) within the TERT gene (6). Together, these considerations will likely inform on the most optimal use of small molecules causing telomere dysfunction, such 6-thio-dG, for the endgame of durable effects in melanoma.

References
Figure Legends

Figure 1: Causing telomere dysfunction to overcome melanoma resistance. Addition of 6-thio-dG causes telomere uncapping. Telomere exposure causes genomic instability leading to the death of targeted and immune checkpoint inhibitor resistant melanoma.
Figure 1:

[Diagram showing Telomere repeats, 6-thio-dG, and Genome instability leading to Therapy-resistant melanoma.]

Telomere repeats

Genome instability

Therapy-resistant melanoma

6-thio-dG

Telomere-binding proteins

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